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## THE RATE OF DEHYDRATION OF COPPER SULPHATE PENTAHYDRATE AT LOW PRESSURES OF WATER VAPOR<sup>1</sup>

BY G. B. FROST AND R. A. CAMPBELL<sup>2</sup>

### ABSTRACT

The rate of dehydration of copper sulphate pentahydrate has been determined over a range of low pressures of water vapor. It has been found that as the pressures are increased, the rate at first drops very rapidly; this drop is followed by a period of marked acceleration, which is followed in turn by a gradual decline. Copper sulphate pentahydrate has been found to be similar in this respect to manganous oxalate dihydrate, which has been hitherto regarded as unique in its behavior. The observed effects are probably due to amorphous to crystalline transitions occurring during induction periods, the durations of which have also been found to depend on water vapor pressure.

### INTRODUCTION

The kinetics of the dehydration of copper sulphate pentahydrate at low pressure has been studied by Garner and Tanner (4), Hume and Colvin (5), Smith and Topley (7), and others. Although these investigations were carried out with different objects in view, and by somewhat different methods, all of them involved, ultimately, the determination of the rate of loss of water per unit area of reaction interface, under conditions where the liberated water molecules were removed very rapidly from the reaction zone. In the determination of these rates, consideration was given to the influence of such factors as the self-cooling of the crystals, and the impedance effect of the layer of reaction product formed around the decomposing crystal. The values obtained are in very satisfactory agreement.

In the course of these studies, Smith and Topley made a careful investigation of the effect of water vapor at low pressures on the rate of dehydration of single crystals of copper sulphate pentahydrate. Crystals, nucleated artificially over their entire surface, were suspended from a quartz spiral balance, and the rate of loss of water determined, either by a static method in which the crystal was exposed to water vapor maintained at a constant pressure, or by a flow method in which hydrogen containing water vapor at a known partial pressure was circulated over the crystal. They found that a gradual, but very marked, decrease in rate occurred as the pressure of water vapor was increased over a range of very low values. At a pressure of 0.04 mm., the rate (expressed as loss in weight per unit area per unit time) was found to be about one-tenth of the rate in vacuum. They did not carry out rate measurements at pressures higher than approximately this value.

<sup>1</sup> Manuscript received August 5, 1952.

Contribution from the Department of Chemistry, Queen's University, Kingston, Ontario.

<sup>2</sup> Present address: Mineral Dressing and Process Metallurgy Division, Department of Mines, Ottawa.

Smith and Topley also pointed out that, for some other hydrated salts, the relationship between rate of dehydration and water vapor pressure might be more complex. A great deal of interest has been shown in one of these cases, namely, the dehydration of manganous oxalate dihydrate, which has been studied independently by Topley and Smith (8) and by Volmer and Seydel (9). Both of these groups of workers used an experimental technique similar to that used by Smith and Topley, and determined the rates of dehydration, both when the dehydration took place in an atmosphere of water vapor at constant pressure, and when the dehydration occurred in an initially evacuated system, the pressure being allowed to build up as the dehydration progressed.

In this case, it was not found possible to express the rate in terms of the loss of water per unit time per unit interfacial area, as had been done for copper sulphate pentahydrate crystals. This was partly due to the fact that the low solubility of this salt hydrate prevented the growth of single crystals large enough to be artificially nucleated over their surface. Consequently, the comparison of rates at various water vapor pressures had to be made by somewhat arbitrary methods. Topley and Smith compared the rates of loss per decigram of material, corresponding to 20% decomposition. Volmer and Seydel compared rates at maximum values read from experimentally determined loss-in-weight versus time curves. The results of the two investigations are in qualitative agreement as regards the shape of the curve obtained. A plot of the experimental values tabulated by Topley and Smith for static experiments at 75.9° C. is shown in Fig. 1.

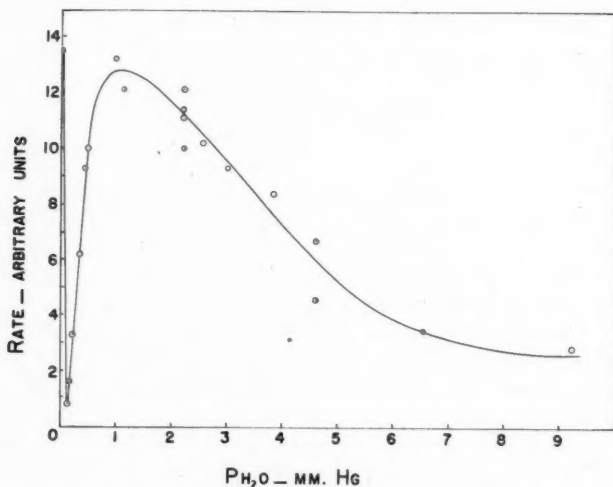


FIG. 1. The dependence of the dehydration rate of aggregates of small crystals of manganous oxalate dihydrate on water vapor pressure (from the data of Topley and Smith).

It will be observed that, as the pressure of water vapor is increased, the rate at first falls in very much the same way as it does with copper sulphate



pentahydrate crystals. This drop, however, is followed by a period of rapid increase to a value comparable to that in vacuum. After passing through a maximum, the rate then gradually declines. The deviation of individual experimental values from the curve drawn is probably due to small differences between the measured pressures and those existing in the actual reaction zone. These deviations are not particularly significant, since it is the qualitative aspect of the curve which is of interest.

Topley and Smith and Volmer and Seydel have given quite different explanations for the over-all aspects of this curve. These need not be discussed here. It will suffice to say that the latter workers found that an amorphous product of relatively high energy content was formed on vacuum dehydration of this salt. They suggested that the formation and ultimate crystallization of this amorphous product is a determining factor in the dehydration mechanism. However, copper sulphate pentahydrate is also known to yield an X-ray amorphous product on low pressure dehydration (6). If the reaction mechanism is to be explained in terms of the role played by such an amorphous intermediate, it might be expected that both salts would have similar relationships in the dependence of dehydration rate on water vapor pressure. The desirability of extending the data for copper sulphate pentahydrate over a wider pressure range has been pointed out by Colvin and Hume (1).

Frost, Moon, and Tompkins (3) have determined the fractional amounts of energy-rich amorphous material formed at a series of dehydration pressures, from copper sulphate pentahydrate and zinc sulphate hexahydrate. Although they did not make kinetic measurements, their results suggest that both of these salts should show the type of rate dependence on pressure which has been observed for manganous oxalate dihydrate. On the basis of their work, the possibility suggests itself that the manganous oxalate dehydration, rather than being unique, may be typical of a group of heterophase reactions in which amorphous intermediates occur.

The purpose of this paper is to report the results of some rate measurements which have been carried out with the foregoing considerations in mind.

#### EXPERIMENTAL METHOD

Experiments were carried out both with finely divided samples and with small single crystals of copper sulphate pentahydrate. To obtain the fine samples, the salt of reagent grade was recrystallized twice and screened, the portion that passed through 60 but not 80 mesh being used. Microscopic examination showed that a fairly large proportion of the aggregate consisted of small well formed triclinic crystals of linear dimensions about 0.2 mm. Other crystals, however, were irregular in form and variable in size. A very rough estimate would indicate that the crystals used were about twice the size of those used by Topley and Smith in their experiments on manganous oxalate dihydrate.

Single crystals, weighing approximately 0.05 gm., were grown slowly from small crystals at room temperature. In the selection of these crystals, those having numerous facets were rejected. The dimensions were measured with

a travelling microscope. The surfaces were activated by rubbing with copper sulphate monohydrate enmeshed in cloth.

The apparatus used did not differ in any important respect from that described in detail by Smith and Topley (7) for their static experiments.

The sample was contained in a small rectangular basket made from 100 mesh copper screen and was suspended from a quartz spiral balance by means of a fine platinum wire. The sensitivity of the spiral was 2.03 mgm. per mm. and was found to be uniform over the extensions used. The spiral was placed in a case of glass tubing 60 cm. long enclosed in a constant temperature cabinet fitted with windows for observation. The spiral case was connected at the top, by a ground joint, to either the vacuum system, or to the source of constant water vapor pressure. It was connected at the bottom, by a ground joint, to the reaction vessel which contained the suspended sample. This reaction vessel was surrounded by a constant temperature bath. Except where otherwise indicated, dehydrations were conducted at a bath temperature of  $40 \pm 0.5^\circ \text{C}$ .

Water vapor at the desired constant low pressures was obtained by adjusting the temperature of ice crystals deposited on glass wool. These were contained in a small bulb connected to the line by a large stopcock. The bulb was immersed in a Dewar flask containing one of several suitable ice-salt eutectic mixtures. Furfural and ethylchloroacetate at their respective freezing points were found suitable in maintaining temperatures below  $-21.0^\circ \text{C}$ . No variation in the pressures so obtained was observed during the time required for experimental readings.

Pressures were read on an oil manometer by means of a cathetometer mounted on a rigid support, the apparatus being assembled so that the same cathetometer could be used for readings of either pressure or spiral extensions. Control experiments, in which one arm of the manometer was connected with the base of the spiral case, indicated that there was no source of error in pressure readings due to pressure gradient through the system.

X-ray powder photographs were made of the dehydration product resulting from all experiments with finely divided crystals.

#### EXPERIMENTAL RESULTS

##### *Preliminary Experiments with Finely Divided Crystals*

Topley and Smith obtained their initial results with manganous oxalate dihydrate by suspending a small weight of fine crystals in a closed and initially evacuated space, and observing both the loss in weight of the crystals and the rise in pressure in the system as the dehydration proceeded. It was considered desirable to carry out this same procedure with copper sulphate pentahydrate crystals. In order to induce nucleation, the crystals were exposed to full vacuum until 10% dehydration to the monohydrate stage occurred. Water vapor pressure was then allowed to build up in the system, and readings of weight loss and pressure taken. The results obtained are shown in Fig. 2.

It will be observed that, following an induction period, the water vapor first accelerates, then retards the net rate of decomposition. By taking the

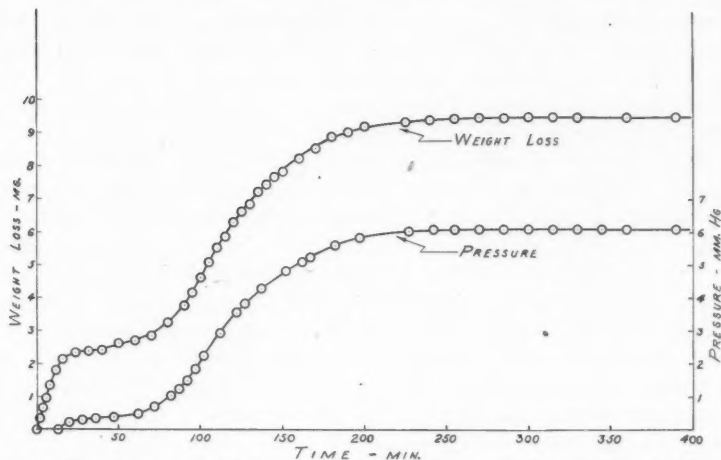


FIG. 2. The change in weight loss, and pressure, during the dehydration of fine crystals of copper sulphate pentahydrate in a closed, initially evacuated space.

slope of the weight loss curve over small intervals, and plotting the values so obtained against the pressure at the mid-points of the intervals, the curve shown in Fig. 3 was obtained.

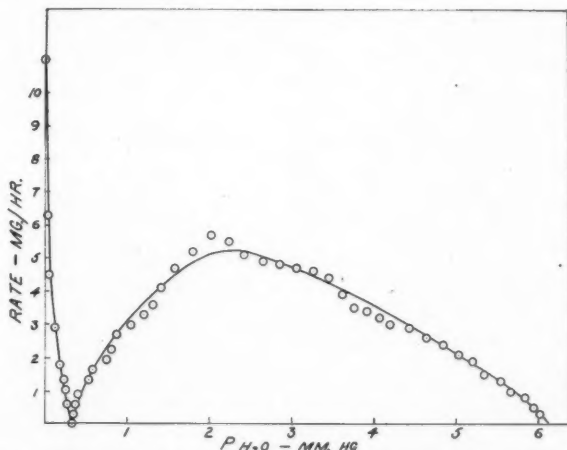


FIG. 3. The dependence of rate of weight loss upon pressure, determined from the data of Fig. 2.

This curve is similar in form to that obtained by Topley and Smith. It is obvious, however, that a similar type of curve would result if either the rate of change of weight loss, or the rate of change of pressure, was plotted against time.

Similar experiments were carried out at several dehydration temperatures.

It was found that, as the temperature of the reacting crystals was lowered, the minimum in rate became sharper and displaced toward the region of lower pressure. Also, the induction period became longer; at 30° C., nearly twelve hours elapsed before an observable increase in pressure or weight loss occurred. At 50° C., the induction period was scarcely observable.

*Experiments with Finely Divided Crystals at Constant Water Vapor Pressures*

A few experiments were carried out under nearly vacuum conditions. In these, the sample was suspended from the spiral, and the system, apart from the spiral case and reaction vessel, evacuated to approximately  $10^{-6}$  mm. The stopcock connecting the spiral case to the vacuum system was then opened and readings started, the pumps being kept in operation throughout the duration of the experiment. After about fifteen minutes, the pressure in the reaction vessel fell to about 0.002 mm. and remained constant at that value.

In experiments conducted at constant pressures of water vapor, the sample was dehydrated as above until 10% decomposition to the monohydrate composition had occurred. The system was then shut off from the pumps, and the sample exposed to the source of water vapor.

In all, 12 experiments were carried out at pressures ranging from 0.002 mm. to 4.57 mm. The weight-loss versus time curves for five of these experiments are shown in Fig. 4, the other curves obtained being entirely similar in type.

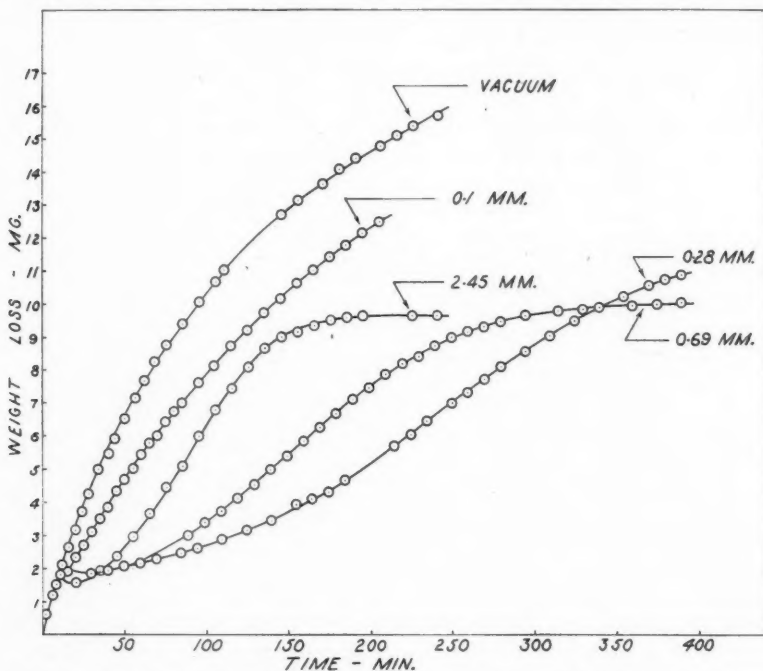


FIG. 4. The change in weight loss of fine crystal aggregates during dehydration at a series of constant water vapor pressures.

The readings recorded cover the entire dehydration period, and include the period of nucleation under vacuum.

It will be observed that these curves fall into two groups. At pressures of 0.002 mm. and 0.1 mm., the rate of loss in weight decreases gradually, and the dehydration proceeds without a break in the rate curve until the monohydrate composition is reached. Furthermore, the rate of dehydration at any given time is lowered with increase in water vapor pressure. X-ray powder photographs have shown that the products obtained under these conditions are amorphous.

The other group of curves are distinctly different in character. They are coincident with the former group up to the point where the pumps are shut off and the system is exposed to water vapor at constant pressure. An induction period then sets in, following which the rates increase over a period of time. The rates then gradually decline, the curves flattening at approximately the trihydrate composition. X-ray powder photographs show trihydrate lines for these end products. The most interesting aspect of this second group of curves lies in the fact that the over-all slopes of the curves in the period following the induction period depend upon the pressure at which the dehydration was conducted. In Fig. 4, these slopes are seen to increase sharply as the pressure is increased from 0.28 mm. to 2.45 mm.

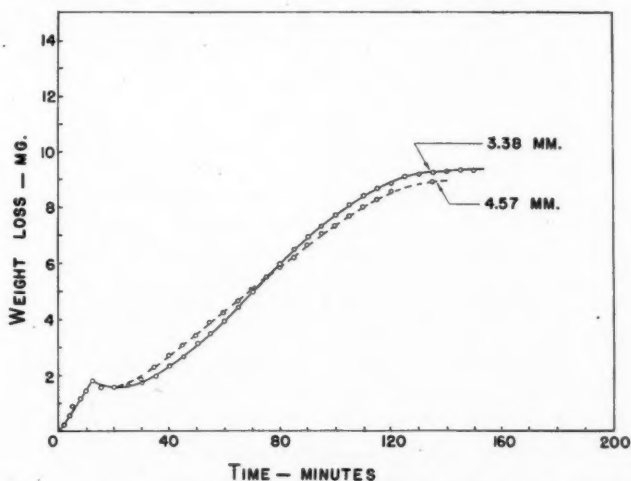


FIG. 5. The change in weight loss of fine crystal aggregates during dehydration in the range of pressure when the rate becomes lowered by pressure increase.

It has been found, however, that there is a limit in pressure beyond which this increase in rate does not occur. This is shown by the two experiments for which data are plotted in Fig. 5. The slope of the portions of the curve following the induction period is lower for the experiment conducted at 4.57 mm. than for that carried out at 3.38 mm. This result has been found to be reproducible both in experiments with finely divided crystals and with single crystals.

In Table I, the rates of dehydration at corresponding water vapor pressures are tabulated. Those given under method *A* were calculated on the basis of the observation that, in all experiments, the loss in weight varies almost linearly with time over a range of fractional decomposition ranging from 0.2 to 0.4 (complete decomposition corresponding to the removal of four water molecules from the pentahydrate lattice, i.e., to a weight loss of 28.8%). This method is very similar to that used for manganous oxalate by Topley and Smith who, as has been mentioned, compared rates corresponding to 20% decomposition.

TABLE I  
DEHYDRATION RATES AT 40° C.

Sample wt., mgm.	Pressure water vapor, mm. Hg	Rate, Method <i>A</i> , mgm./hr./dgm.	Rate, Method <i>B</i> , mgm./hr./dgm.	X-ray powder photograph
62.0	0.002	10.53	19.38	No lines
62.0	0.10	6.84	7.90	No lines
62.0	0.28	3.15	3.65	Trihydrate lines
62.3	0.69	4.03	4.11	Trihydrate lines
62.0	0.69	4.16	4.46	Trihydrate lines
62.0	1.18	3.74	4.37	Not determined
62.0	1.22	4.21	4.68	Trihydrate lines
62.0	2.45	7.32	7.75	Trihydrate lines
62.0	2.62	7.85	8.33	Trihydrate lines
62.0	3.38	9.36	9.88	Not determined
62.0	4.57	8.15	8.30	Not determined
62.2	4.57	8.30	8.50	Trihydrate lines

The rates tabulated under method *B* were determined for each curve by finding the slope over small intervals and determining the point of inflection. In other words, the rates so tabulated are those prevailing at the point where the rate begins to decline owing, presumably, to the impedance effect.

Both of these methods are arbitrary, and are particularly so when applied to the two curves which show a gradual decrease in rate throughout. However, for these curves the only feature which is of immediate concern is that the rate drops rapidly with increasing pressure.

The rates determined by methods *A* and *B* are shown plotted against corresponding pressures in Fig. 6 and Fig. 7 respectively.

A comparison of these curves with that shown in Fig. 1 shows clearly that the effect of increasing water vapor pressure on dehydration rate is qualitatively similar in the two cases.

#### *Experiments with Single Crystals*

A series of experiments were carried out in which single crystals weighing approximately 50 mgm. were subjected to the procedures which have been described.

When nucleated crystals were placed in an initially evacuated space and allowed to dehydrate while the pressure increased, the results obtained were similar to those shown in Fig. 3.

Dehydrations at constant pressure were carried out over a range of pressures



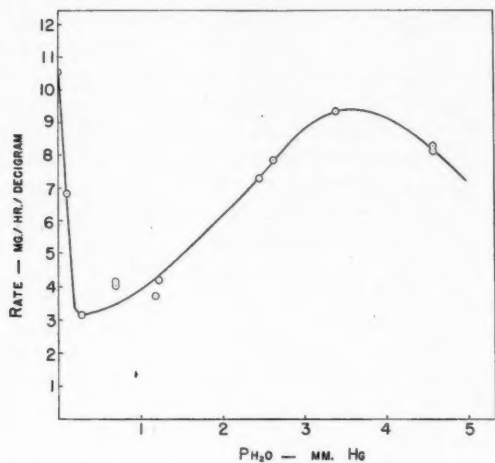


FIG. 6. The dependence of rate upon pressure (method A).

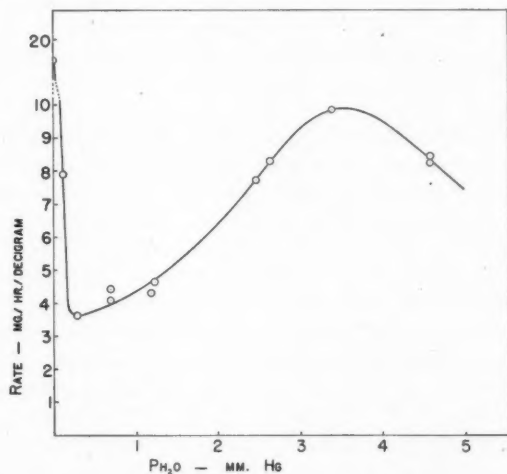


FIG. 7. The dependence of rate upon pressure (method B).

from nearly vacuum to 4.60 mm. The results of four experiments are shown in Fig. 8. It will be seen that the rates are very much slower, the dehydration extending over two days in some cases.

These experiments were carried out with the hope that the rates might be expressed as loss in weight per unit time per unit area, the interfacial areas at different stages of decomposition being calculated from the linear dimensions of the crystal by making the assumption that the reaction is propagated inwards in a uniform manner.



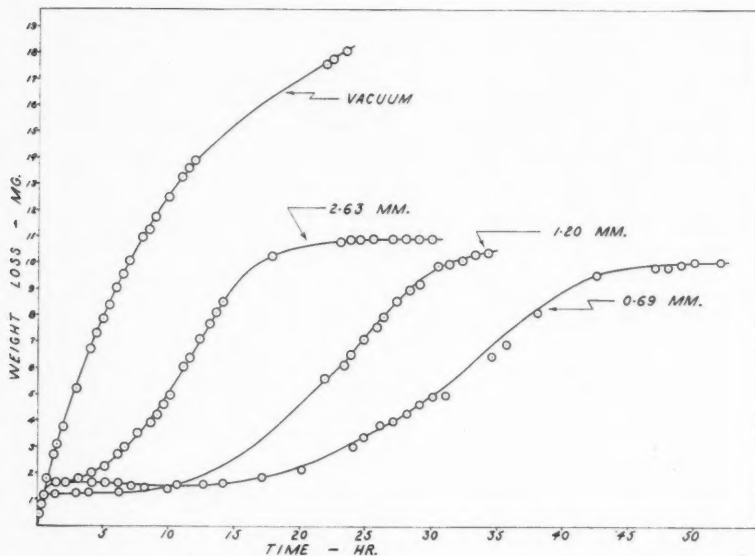


FIG. 8. The change in weight loss of single crystals during dehydration at a series of constant water vapor pressures.

In order to test this assumption the following experiment was carried out. Several small crystals were prepared and selected as has been described. These were placed in a small tube which was connected to the vacuum system through a ground joint and a large stopcock. The tube was surrounded by a Dewar flask containing water at 40° C. A crystal was also placed on the spiral pan housed in the spiral case as has been described. The water vapor pressure was then adjusted to one of a series of values. The crystals in the tube and the crystal on the spiral pan were therefore subjected to identical conditions of temperature and pressure. At suitable intervals during the dehydration periods, the proper stopcocks were closed, the system opened, and one of the crystals removed for microscopic examination. The tube was then replaced, the air pumped out, the sample again exposed to the water vapor for a definite time, and a second crystal removed, the whole operation being then repeated a number of times. Since constant conditions were maintained, it was assumed that the loss in weight of any one of the crystals in the tube corresponded to that of the crystal on the spiral basket.

Microscopic examination of crystals dehydrated to various stages under nearly vacuum conditions confirmed the findings of previous workers that the reaction proceeds at an interface which moves into the crystal as the reaction proceeds. Fairly satisfactory correlation was obtained between the percentage weight loss as determined from the spiral extension and that estimated from the position of the interface. In view of the experiments of Smith and Topley, who were able to calculate their rates to zero impedance, it would appear

likely that dehydrations carried out at pressures to the left of the minimum shown in Fig. 6 or in Fig. 7 are of this type.

However, microscopic examination of crystals dehydrated to various stages at pressures greater than those corresponding to the minimum rate gave no evidence of such correlation. In most cases the position of the interface could be estimated only in the very early stages of decomposition, and all traces of an interface disappeared long before the decomposition was complete. It appeared from these experiments that nucleation developed through the entire crystal very shortly after the decomposition commenced, probably during the induction period. This marked difference in behavior is of interest and is undoubtedly related to the distinct difference in character between the types of dehydration curves to which reference has been made.

In view of these experiments, it was necessary therefore to evaluate rates by the methods used in the case of finely divided crystals. When these rates were plotted against pressure, curves were obtained similar to those shown in Fig. 6 and Fig. 7.

#### DISCUSSION

The similarity in rate-pressure dependence, which these experiments have shown to exist for manganous oxalate dihydrate and copper sulphate pentahydrate, clarifies our understanding of the processes involved to the extent of removing the difficulty in interpretation which has arisen as a consequence of regarding these salt hydrates as being different in behavior. The comment which follows is intended to bring into relief certain features which we regard as pertinent, rather than to suggest explanations which differ in any fundamental way from those which have been already offered (1, 8, 9).

We believe that the key to the interpretation of the observed dependence of rate upon pressure shown in Figs. 6 and 7 lies in the fact that the durations of the induction periods shown in Figs. 4 and 8 are also pressure dependent, and furthermore, that the curves are very nearly linear following these induction periods. These curves show qualitatively, but clearly, that the induction periods become shorter as the pressure becomes greater. This is also the case in the experiments shown in Fig. 5 although the subsequent rates show an inverse relationship with pressure.

Experiment has shown that the breakdown of the copper sulphate pentahydrate lattice at low pressure results in the formation of a product which does not diffract X rays. This product is relatively stable. It may be prepared in the laboratory, and some of its properties studied. However, its exact nature is as yet unknown. Experiment shows that the orientation of this amorphous product into a stable lattice occurs in the presence of traces of water vapor (3).

Upon low pressure dehydration, when water molecules are continually drawn away from the reaction zone, the amorphous product has the stoichiometrical composition of the monohydrate. The group of weight-loss versus time curves obtained at very low pressures represent dehydrations of this type. The pentahydrate lattice breaks down into amorphous monohydrate, the

gradual decrease in rate being due to increasing impedance and diminishing interfacial area.

The same process must be assumed to occur in all other cases up to the point where the sample is exposed to water vapor at constant pressure. At this stage (following in some cases a slight increase in weight due to adsorption) the induction period sets in during which there is little observable change in weight. It has been pointed out that microscopic examination of crystals dehydrated to this stage shows the reaction zone to have advanced far into the crystal. It is probable, therefore, that during the induction period the pentahydrate lattice continues to collapse into the amorphous product, but that the liberated water molecules are retained in the amorphous layer in sufficiently large numbers to make the weight loss undetectable by the method used. As the water vapor pressure in the region external to the crystal is increased, the pressure gradient between the crystal and its surroundings is reduced. Hence the rate of removal of water molecules is lowered and the effectiveness in inducing crystallization is increased.

When crystallization occurs, the escape of water molecules will be facilitated by a decreased adsorption and through the development of spaces between the small crystals through which water molecules may pass. The induction period will be followed, therefore, by a period during which water molecules will escape at a nearly linear rate, this rate being eventually decreased as the impedance effect of the crystalline layer increases. This, of course, is what has been observed in the loss-in-weight versus time curves which have been reported in the foregoing.

On the basis of this view, the center of interest shifts from the effect of pressure on the rate following the induction period, to the effect of pressure on the induction period itself. In other words, the rate of escape of water molecules from the reaction zone will be increased in proportion to the extent to which crystallization has occurred. But this extent of crystallization will depend upon a diminishing pressure gradient, or consequently upon increase of external water vapor pressure. It is interesting to note that in those experiments in which crystals were allowed to dehydrate into a closed and initially evacuated space, the long induction period observed at 30° C. is practically nonexistent at 50° C. In other words, at the higher temperature, water vapor is produced rapidly enough to cause rapid crystallization of the amorphous product and the consequent release of water molecules from the reaction zone.

While these considerations may be helpful in arriving at a qualitative explanation of the results obtained, it must be emphasized that the observed changes in rate occur with changes in external pressure of only 2 or 3 mm. This sensitivity to water vapor over this small range is very surprising. A more definite knowledge of the nature of the amorphous product might throw light on the nature of the processes of nucleation and crystallization which appear to be dependent upon these small changes in pressure.

Mention should also be made of the fact that under the experimental conditions, in which dehydrations were conducted at a definite static pressure of water vapor, the end crystalline product was trihydrate instead of mono-

hydrate as might have been expected. Frost, Moon, and Tompkins (3) carried out dehydrations over substantially the range of pressures used in this work, and obtained mixtures of crystalline pentahydrate along with crystalline and amorphous monohydrate and with only slight evidence of trihydrate formation. However, their dehydration pressures were obtained by means of a throttled stopcock device, under which condition there would be a movement of water molecules away from the reaction interface.

The stability of the trihydrate phase at pressures far below those corresponding to the tri-monohydrate equilibrium (16 mm. at 40° C.) has been observed by other workers and is consistent with the finding of Cooper, Colvin, and Hume (2) that the rates of nucleation and propagation of the trihydrate are much slower than those of the pentahydrate. From this point of view, the curves of Figs. 4 and 8 level off at the induction period of the crystalline trihydrate to crystalline monohydrate transition.

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# THE FISSION YIELD OF $I^{131}$ IN THE THERMAL NEUTRON FISSION OF $U^{235}$ <sup>1</sup>

BY ROSALIE M. BARTHOLOMEW, F. BROWN, R. C. HAWKINGS,  
W. F. MERRITT, AND L. YAFFE<sup>2</sup>

## ABSTRACT

The fission yield of  $I^{131}$  has been investigated using improved extraction techniques and disintegration rate measurements. The value obtained for the fission yield is  $(3.1 \pm 0.1)\%$ . This is relative to a  $Ba^{140}$  reference fission yield of 6.1%. The half-life for  $I^{131}$  has been determined and found to be  $8.05 \pm 0.01$  days.

## INTRODUCTION

Recent reports (16, 11) of fine structure in the fission yield - mass curve obtained from thermal fission of  $U^{235}$  have made advisable a redetermination by improved radiochemical techniques of the yields of some of the nuclides formed in fission. The mass 131 chain has been used by Thode *et al.* (16) as their standard and thus an accurate knowledge of the chain yield is necessary. This paper deals with an improved determination of the yield of the last radioactive member of this chain,  $I^{131}$ .

All previous measurements of the fission yield of  $I^{131}$  have been with reference to the yield of  $Ba^{140}$ . The normalization values assumed vary from worker to worker. For comparison purposes, we have normalized all earlier values for  $I^{131}$  to a  $Ba^{140}$  yield of 6.1% (9). This is currently believed to be the best value and has been used as the reference in this work.

On this basis the following results have been obtained by other investigators: Anderson, Fermi, and Grosse (2)—2.4%, Grummitt and Wilkinson (13)—1.4%, Yaffe and Mackintosh (21)—2.4%, Engelkemeir, Seiler, Steinberg, and Winsberg (7)—2.8%.

It has recently become evident that the fluctuations and possibly low values quoted above can be improved by better chemical extraction methods and better disintegration rate measurements, both of which were used in this work.

The nuclide  $I^{131}$  was first identified by Livingood and Seaborg (15) and first found as a fission product by Abelson (1). Recent values for the half-life are  $8.02 \pm .03$  days by Sinclair and Emery (18) and  $8.1409 \pm .0062$  days by Sreb (19), although no experimental details have been published of Sreb's work.

The  $I^{131}$  decay scheme is extremely complex and is still a matter of some dispute, although probably the best scheme is that recently proposed by Bell and Graham (3). Owing to the complexity of the decay scheme and the fact that about 1% of the  $I^{131}$  disintegrations go to a metastable state of  $Xe^{131}$  with a half-life of about 12 days (5), the accurate measurement of the disintegration rate presents a problem.

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<sup>2</sup> Present address: Department of Chemistry, McGill University, Montreal, Quebec.

## EXPERIMENTAL

*(a) Irradiation*

Accurately weighed samples (10–20 mgm.) of uranyl nitrate hexahydrate were irradiated in sealed quartz ampoules in the NRX nuclear reactor. Separate samples were used for the  $Ba^{140}$  and  $I^{131}$  determinations. The ampoules were arranged so that they were very close together and thus received the same neutron irradiation. The samples used were of such a size that self-shielding was negligible.

The position chosen in the reactor was one where the neutrons were predominantly thermal. The length of irradiation was 48 hr. in two experiments and 96 hr. in the third.

*(b) Chemical Extraction**(i) Iodine*

The iodine was extracted from the irradiated uranium about one week after the end of irradiations. This allowed the  $Te^{131}$  parent of the  $I^{131}$  to decay essentially completely.

The chemical separation method used was one which had been shown to give complete interchange between the radioactive iodine and the added carrier. The method is essentially that of Glendenin and Metcalf (10) which has been modified slightly by Yaffe, Day, and Greer (20). The iodine carrier is added as KI to an alkaline solution of the uranium salt. The KI is oxidized to  $I^{+7}$  with sodium hypochlorite, reduced to  $I_2$  with hydroxylamine hydrochloride, further reduced to  $I^-$  with sulphur dioxide, and oxidized to  $I_2$  with sodium nitrite – nitric acid mixture. This cycle was repeated several times, finally leaving the iodine in the reduced state. Full details are given in Reference 20. The final reduced solution was made slightly alkaline with lithium hydroxide and made up to a standard volume.

*(ii) Barium*

The barium was extracted by a method described previously (12). Using this method the barium was separated from the uranium and other fission products by several chloride, chromate, and nitrate precipitations with the addition of appropriate hold-back carriers. The final barium nitrate precipitate was dissolved in water and made up to a standard volume. Aliquots were taken from this for measurement purposes.

*(c) Source Preparation for Measurement*

From the master solution, aliquots were pipetted out with calibrated micro-pipettes and deposited on thin films. The area over which the source was to be spread was previously wetted by placing on it and then removing a drop of 5% insulin. The films used were of laminated Zapon-formvar (100  $\mu$ gm. per  $cm.^2$ ) sputtered with 25  $\mu$ gm. per  $cm.^2$  gold on either side.

*(i) Barium*

The aliquot of the barium nitrate solution was placed on the film and dried using an infrared lamp. This gave essentially a "weightless" source, i.e., self-absorption was negligible.

*(ii) Iodine*

The aliquot of iodide solution was added to a solution of silver nitrate already



present on the film. Approximately two equivalents of  $\text{Ag}^+$  were used per equivalent of  $\text{I}^-$ . This excess usually resulted in the precipitation of some silver oxide but has been found to give a more stable precipitate of silver iodide. Attempts to reduce the silver concentration resulted in loss of radioactive iodine. A good source appears uniformly gray to black on the film—all others were discarded.

A sample on the film was heated for 15–20 min. prior to each measurement using a 250 watt infrared lamp at a distance of 15–20 cm. This technique was adopted to get rid of the xenon decay products formed from the iodine. This is not too important with  $\text{I}^{131}$  since only about 1% decays to an active xenon daughter. All other iodine products had decayed before extraction. This is very important, however, in the measurement of the other iodine isotopes and this technique will be described in a forthcoming publication.

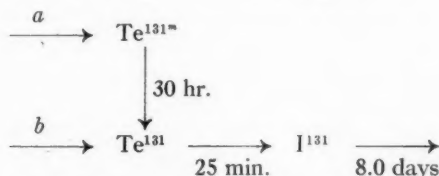
#### (d) Measurements

All activities were measured using the  $4\pi$   $\beta$ -counting technique as described by Borkowski (4) and Hawkings, Merritt, and Craven (14). This counter has the advantage of very great stability, low resolving time, and does not require the use of major correction factors. Small corrections, of the order of one per cent, are made for coincidence losses and for loss of  $\beta$  particles in the film holding the sample. These corrections can be made with good accuracy.

#### (e) Radioactive Growth and Decay

Decay curves for  $\text{I}^{131}$  were followed for many half-lives in subsidiary experiments as shown in Table II and a very accurate value of the half-life obtained. Each half-life shown represents a separate irradiation and extraction. The activity in the iodine sample after extraction consisted principally of  $\text{I}^{131}$  since the other iodine isotopes had almost completely decayed away.

(i)  $\text{I}^{131}$  is formed as a member of the following chain



where  $a$  and  $b$  are the fractional yields of  $\text{I}^{131}$  from the parent isomers.

$$a/b = 87/13 \quad (8)$$

If the sample of uranium is irradiated for a time  $t_0$  and then allowed to decay for a time  $t_1$  (measured from the end of irradiation), then the number of atoms of  $\text{I}^{131}$ ,  $N_1$ , present at time  $t_1$  can be shown to be

$$\begin{aligned}
 N_1 = & Y_{131} N \left\{ \left( \frac{b+a}{\lambda_3} \right) (e^{-\lambda_3 t_1} - e^{-\lambda_3 (t_0+t_1)}) \right. \\
 & + \frac{a\lambda_2}{(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)} (e^{-\lambda_1 t_1} - e^{-\lambda_2 t_1} - e^{-\lambda_1 (t_0+t_1)} + e^{-\lambda_2 (t_0+t_1)}) \\
 & \left. + \left( \frac{b+a}{\lambda_3 - \lambda_2} - \frac{a\lambda_2}{(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_2)} \right) (e^{-\lambda_2 t_1} - e^{-\lambda_3 t_1} - e^{-\lambda_2 (t_0+t_1)} + e^{-\lambda_3 (t_0+t_1)}) \right\}
 \end{aligned}$$



where  $Y_{131}$  = fission yield of  $I^{131}$ ,

$N$  = fission rate,

$\lambda_1$  = decay constant of  $Te^{131m}$  (30 hr.),

$\lambda_2$  = decay constant of  $Te^{131}$  (25 min.),

$\lambda_3$  = decay constant of  $I^{131}$ .

This is based on the reasonable assumption that the independent fission yield of  $I^{131}$  is negligibly small.

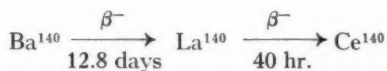
The above equation may be expressed in the following form

$$N_I = Y_{131} N k_1$$

where the value of  $k_1$  may be calculated from the experimental data and known constants involved.

Then the disintegration rate is  $N_I \lambda_3 = Y_{131} N k_1 \lambda_3$ .

(ii)  $Ba^{140}$



Since the daughter of the  $Ba^{140}$  is shorter-lived than the parent, a "growth" curve rather than a decay curve results if one plots the ratio  $(Ba^{140} + La^{140})_t / (Ba^{140})_{t_0}$  against time. The symbols  $Ba^{140}$  and  $La^{140}$  refer to the disintegrations per unit time from these nuclides, while  $t$  and  $t_0$  refer to the actual time of measurement and the time of separation of the  $Ba^{140}$  from the  $La^{140}$  respectively;  $t_0$  is the actual time of the last barium nitrate separation. The growth curve was plotted using the best values available for the half-lives.

The half-life of  $La^{140}$  was checked experimentally using neutron-irradiated 'spec-pure' lanthanum oxide. A value of  $40.2 \pm 0.1$  hr. was obtained by a least squares fit over 12 half-lives. Source reproducibility was guaranteed by taking repeated aliquots from a master solution. Special precautions were taken to prevent evaporation of the master solution. A value of 12.8 days (6) was taken for the half-life of  $Ba^{140}$ .

Using the theoretical growth curve based on the above half-lives, the disintegration rate of  $Ba^{140}$  at the time of separation was calculated. The constancy of this value, as shown in Table I, where observations were made over a wide variation in the ratio  $La^{140}/Ba^{140}$ , shows that the soft radiations of  $Ba^{140}$  were counted with good efficiency.

The disintegration rate of  $Ba^{140}$  was then corrected back to the end of irradiation using a half-life of 12.8 days. This then gives

$$N_{Ba} \lambda_{Ba} = Y_{140} N$$

where  $N_{Ba}$  and  $\lambda_{Ba}$  are the number of atoms of  $Ba^{140}$  and its decay constant respectively.  $Y_{140}$  is the fission yield of  $Ba^{140}$  and  $N$  again the fission rate. Using this, the fission yield of  $I^{131}$  ( $Y_{131}$ ) is calculated as follows

$$Y_{131} = \frac{(N_I \lambda_3) Y_{140}}{k_1 \lambda_3 (N_{Ba} \lambda_{Ba})}$$

(f) Chemical Yields

The chemical yields were determined by gravimetric analyses of the solutions.

Silver iodide and barium sulphate were precipitated, using standard analytical methods.

## RESULTS

TABLE I

Time elapsed between separation and measurement ( $t - t_0$ ) in hours	Theoretical ratio of $\text{La}^{140}/\text{Ba}^{140}$	$\text{Ba}^{140}$ concentration at $t_0$ (dis./sec./ml. $\times 10^{-3}$ )
4	0.07	4.76
27	0.39	4.77
140	1.01	4.76
800	1.15	4.77
1650	1.15	4.78

The results of the half-life determinations are given in Table II.

TABLE II

Period of measurement (days)	Weighting factor	Observed half-life (days)
27	1.000	8.058
27	1.000	8.068
27	1.000	8.050
50	1.852	8.062
69	2.556	8.055*
35	1.296	7.980

\*This sample of  $\text{I}^{131}$  was not obtained from the fission of  $\text{U}^{235}$ , but was prepared by the neutron irradiation of tellurium.

The results were weighted using factors directly proportional to the period over which the measurement took place. The observed half-lives shown are the best values obtained by a least squares fit.

The weighted average for the half-life of  $\text{I}^{131}$  is  $8.05 \pm .01$  days. Owing to the long period of time taken for the measurements, the care to avoid any  $\text{Xe}^{131}$  contribution, and the stability of the equipment used, this value is considered to be reliable.

The fission yield data are shown in Table III. The value obtained is  $(3.10 \pm .01)\%$  relative to a yield of 6.1% for  $\text{Ba}^{140}$ .

TABLE III

Expt. No.	Length of irradiation, $t_0$ (hr.)	Time between end of irradiation and chemical separation, $t_1$ (hr.)		Dis./sec./mgm. uranyl nitrate at time of separation		Fission yield in per cent (relative to $\text{Ba}^{140} + 6.1\%$ )
		$\text{Ba}^{140}$	$\text{I}^{131}$	$\text{Ba}^{140}$	$\text{I}^{131}$	
1	48.0	126.3	123.0	$3.500 \times 10^5$	$1.840 \times 10^4$	3.10
2	48.0	179.8	207.0	$4.108 \times 10^5$	$1.585 \times 10^4$	3.12
3	96.0	111.0	253.0	$4.640 \times 10^5$	$2.840 \times 10^4$	3.09
Average						$3.10 \pm .01$

It is exceedingly difficult to assess any systematic errors which may be carried along. We are suggesting a value of  $(3.1 \pm 0.1)\%$  for the  $I^{131}$  fission yield. The error quoted is 10 times the standard deviation and is probably greater than any systematic error present. This agrees well with a recent unpublished value of 3.0% obtained by Pappas and Coryell (17).

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**THE BIOGENESIS OF ALKALOIDS**  
**VII. THE FORMATION OF HORDENINE AND N-METHYLTYRAMINE FROM**  
**TYROSINE IN BARLEY<sup>1</sup>**

BY EDWARD LEETE<sup>2</sup> AND LÉO MARION

ABSTRACT

Feeding *dl*-tyrosine-2-C<sup>14</sup> to sprouting barley resulted in the formation of radioactive hordenine and N-methyltyramine in the roots. Isolation and degradation of these alkaloids showed that all the activity was located in the  $\alpha$ -carbon atom of the side chain, thus indicating that tyrosine is a precursor of N-methyltyramine and hordenine.

It has been shown previously (6) that tyramine is a precursor of hordenine in sprouting barley, the hordenine being formed by stepwise methylation via N-methyltyramine (4). It seemed highly likely that the source of tyramine in the roots was tyrosine, since it is well established that this conversion can be brought about by a variety of bacteria and by the kidney tissue of animals.

This hypothesis was tested by feeding *dl*-tyrosine-2-C<sup>14</sup> to sprouting barley and then, after a suitable period of time, isolating the N-methyltyramine and hordenine from the roots. These alkaloids were found to be radioactive and degradation as previously described (6) showed that all the activity of both alkaloids was located in the  $\alpha$ -carbon atom of the side chain (see Table I). The higher activity of the N-methyltyramine was again observed, indicating a stepwise methylation. Since no tyramine could be detected in the extracts from the roots, it thus seems that tyramine has only a transient existence in the normal growth of the roots. It is immediately methylated to N-methyltyramine, while further methylation to hordenine takes place more slowly.

The source of hordenine in the roots of barley has now been clearly established, but this can only be considered as a preliminary step in determining the function of this alkaloid in the physiology of the plant.

The indole alkaloid gramine is produced in the leaves of the strain of barley used in this investigation (Charlottetown No. 80) and in view of the recent suggestion of Robinson (7) that tyrosine may be a source of indole we examined the leaves of the sprouting barley which had been fed with the radioactive tyrosine. The leaves were found to be radioactive, as also was the methanol extract of the leaves. However, the crude gramine isolated from the extract was found to be completely inactive. It has been shown that tryptophane is a precursor of gramine (1) and other workers have shown that tryptophane is synthesized by the condensation of indole and serine in *Neurospora* (8, 9, 10). Thus, had the tyrosine-2-C<sup>14</sup> been converted to indole by Robinson's mechanism, gramine should have been obtained in which radioactivity was located in the  $\alpha$ -position of the indole nucleus. At least this would be the case if it be assumed that any indole produced would be converted to

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<sup>2</sup> Holder of a Postgraduate Travelling Fellowship from the Goldsmiths' Company of London, England.

tryptophane and thence to gramine. The biosynthesis of tryptophane in barley leaves is being investigated.

#### EXPERIMENTAL

##### *Preparation of dl-Tyrosine-2-C<sup>14</sup>*

C<sup>14</sup>-Methyl labelled sodium acetate (1.64 gm.) was converted to bromoacetic acid by the method of Fields *et al.* (2). The crude bromoacetic acid was dissolved in water (5 ml.) and sodium carbonate (1.2 gm.) added to the solution. The mixture was warmed to 60° and after the addition of sodium cyanide (1.1 gm.) dissolved in a little water, maintained at 60° for 15 min., and then refluxed for five minutes. The resulting brown solution was cooled to 0°, concentrated hydrochloric acid (2.5 ml.) added, and the solution evaporated to dryness *in vacuo*. The residue was extracted with absolute alcohol and the filtered extract on evaporation yielded crystalline cyanoacetic acid. The crystalline acid was suspended in ether and to the suspension was added a solution of excess diazoethane in ether; after the solution had stood for five minutes it was filtered and the ether evaporated off on the water bath. The ethyl cyanoacetate-2-C<sup>14</sup> thus obtained was a yellow oil. It was used to synthesize *dl*-tyrosine-2-C<sup>14</sup> by the method of Fields *et al.* (3). The yield of *dl*-tyrosine was 1.826 gm. (50.3% based on sodium acetate).

##### *Administration of dl-Tyrosine-2-C<sup>14</sup> to the Barley*

Barley (Charlottetown No. 80, 720 gm.) was grown as previously described (4). On the sixth day of sprouting a solution of *dl*-tyrosine-2-C<sup>14</sup> (506.75 mgm.) in distilled water (600 ml.) containing concentrated hydrochloric acid (0.40 ml.) was fed to the barley. The administered tyrosine had a specific activity of  $3.31 \times 10^4$  disintegrations per minute per millimole and a total activity of  $9.40 \times 10^7$  disintegrations per minute. The low pH of the solution had no apparent effect on the growth of the barley and after one day in contact with the roots the nutrient solution was neutral.

##### *Isolation of the Alkaloids from the Roots*

On the 11th day of sprouting the shoots and roots of the barley were separated. The crude alkaloids from the roots were obtained by established methods (5, 6). The roots (80.4 gm.) which had been extracted with hot methanol for 48 hr. still had a high residual activity of 765 disintegrations per minute per mgm. Paper chromatography of the crude extract (6) followed by a survey of the radioactivity of the developed chromatogram showed that the activity of the extract was located in spots coincident with N-methyltyramine and hordenine detected by Millons reagent. There was no trace of radioactivity in the position where tyramine would occur on the chromatogram. Chromatography of the extract on alumina (6) yielded hordenine (0.0996 gm. with a specific activity of  $1.61 \times 10^5$  disintegrations per minute per millimole) and N-methyltyramine (0.0678 gm. with a specific activity of  $6.12 \times 10^5$  disintegrations per minute per millimole). Both the hordenine and N-methyltyramine were diluted with inactive alkaloids, and subsequent degradations were carried out with these diluted samples. Degradations were carried out as previously

described and the specific activities of the derivatives and degradation products of the alkaloids are summarized in Table I.

TABLE I

Derivative <sup>3</sup>	Disintegrations per minute per millimole <sup>4</sup>	
	From hordenine	From N-methyltyramine
Alkaloid	$1.61 \times 10^5$	$6.12 \times 10^5$
Picrate	$1.69 \times 10^5$	$6.72 \times 10^5$
Hydrochloride	$1.70 \times 10^5$	$6.38 \times 10^5$
Platinochloride	$2 \times 1.69 \times 10^5$	$2 \times 6.07 \times 10^5$
Hordenine methiodide	$1.57 \times 10^5$	$6.38 \times 10^5$
O-Methylhordenine methiodide	$1.62 \times 10^5$	$6.07 \times 10^5$
Trimethylamine platinochloride <sup>5</sup>	0	0
Anisic acid <sup>6</sup>	0	0
Homoanisaldehyde oxime <sup>7</sup>	$1.71 \times 10^5$	$6.57 \times 10^5$
Anisic acid <sup>8</sup>	$1.60 \times 10^5$	$6.10 \times 10^5$

<sup>3</sup> The preparation of all the derivatives was carried out exactly as described previously (6).

<sup>4</sup> The radioactive measurements were determined on thin samples, making the usual corrections for self-absorption, etc.

<sup>5</sup> Obtained from the Hofmann degradation of the O-methylhordenine methiodide.

<sup>6</sup> Obtained by the oxidation of *p*-vinylanisole from the Hofmann degradation.

<sup>7</sup> Obtained by oxidation of the *p*-vinylanisole with mercuric oxide and iodine.

<sup>8</sup> Obtained by oxidation of the homoanisaldehyde oxime.

#### Isolation of Gramine from the Shoots

The shoots (55.75 gm.) which had been dried at room temperature were extracted as previously described (1). The residue (28.8 gm.), obtained by evaporation of the crude methanol extract, was assayed and found to have an activity of 34.6 disintegrations per minute per mgm., or a total activity of  $1.00 \times 10^6$  disintegrations per minute. Crude gramine (0.281 gm.) isolated from the extract by the usual method (1) was found to be inactive.

#### ACKNOWLEDGMENT

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# THE REACTION OF ACTIVE NITROGEN WITH ACETYLENE<sup>1</sup>

By J. VERSTEEG<sup>2</sup> AND C. A. WINKLER

## ABSTRACT

The main products of this reaction were hydrogen cyanide and polymer that contained approximately 32% nitrogen. Yields of these substances increased to constant values with increase of acetylene flow rate. Some cyanogen and methane were also formed. The yield of cyanogen passed through a maximum with increased flow rate of acetylene, but the methane yields were quite erratic.

Previous studies in this laboratory have served to outline the main features of several reactions involving the attack of active nitrogen on paraffin and olefine hydrocarbons (1, 3, 6, 8). The present paper summarizes the results of a similar study with acetylene, with an interpretation in terms of atomic nitrogen as the reactive species in active nitrogen (cf. 6).

## EXPERIMENTAL

The apparatus and general experimental and analytical techniques were identical with those described in the earlier papers. Hydrogen cyanide and cyanogen were estimated by an adaptation of the method of Wallis (9), acetylene by absorption in potassium iodomercurate (4), and methane and hydrogen by combustion (2).

## RESULTS AND DISCUSSION

The reaction was accompanied by a very bright flame, more reddish in color than similar flames with other hydrocarbons studied in this laboratory. The size of the flame was unaffected by hydrocarbon flow rate in the range used, and the flame was always located in the immediate vicinity of the hydrocarbon jet. The temperature indicated by a thermocouple in the center of the reaction vessel was approximately 165°C., but the true reaction (flame) temperature was probably much higher than this value.

The main products were hydrogen cyanide and a brown polymer that accumulated, for the most part, on the wall of the reaction vessel. A small amount of polymer of darker color was also deposited in two consecutive liquid nitrogen traps in the main pumping line. No ethylene or ethane was detected among the products.

Preliminary experiments showed that formation of polymer in one experiment reduced the amount of reaction in a subsequent experiment, presumably because the concentration of nitrogen atoms available for reaction with acetylene was reduced by their reaction with polymer. Prior to each experiment, the apparatus following the discharge tube was therefore cleaned thoroughly with chromic acid, followed by water, potassium hydroxide solution, and copious amounts of water. It was then poisoned with dilute phosphoric acid solution in the usual way.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Que. Grateful acknowledgment is made to the Defence Research Board for financial assistance and permission to publish.

<sup>2</sup> Holder of a Research Council of Ontario Scholarship.



The yields of the various products as a function of the flow rate of acetylene are shown in Fig. 1.\* At very low flow rates of acetylene, corresponding to at

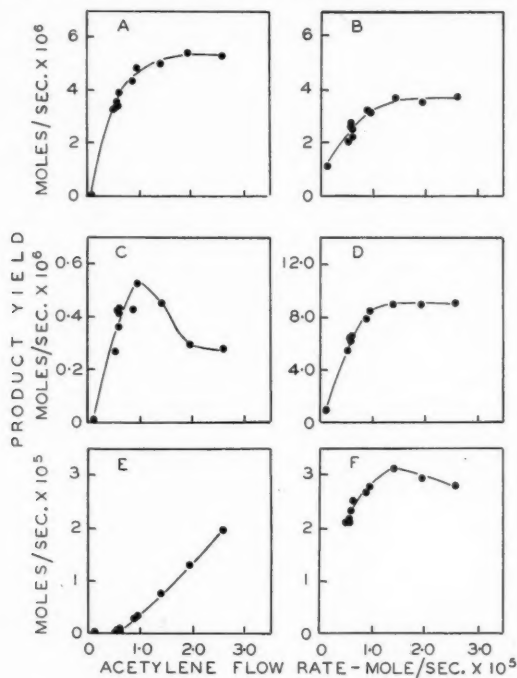


FIG. 1. Relation between yields of products and flow rate of acetylene.

Curve A—hydrogen cyanide.

Curve B—polymer (plotted as acetylene lost).

Curve C—cyanogen.

Curve D—total nitrogen reacted.

Curve E—acetylene (recovered).

Curve F—total hydrogen in noncondensable gases (i.e. as H<sub>2</sub> and CH<sub>4</sub>).

least a sevenfold excess of nitrogen atoms, negligible yields of hydrogen cyanide, cyanogen, or methane were obtained. The acetylene that reacted at these flow rates presumably appeared almost entirely as polymer. From appropriate carbon balances for the system at various flow rates, the curve for polymer formation (Fig. 1B) was plotted.

The identity of the polymer has not been established with any certainty. From its rather mottled color it may be inferred that it was not entirely homogeneous. Two samples, one from the reaction vessel, the other from the traps, were obtained by washing some of the more loosely held polymer from the glass surface with distilled water. Dumas nitrogen analyses\*\* showed that the material from the

\*The maximum yield of cyanogen corresponds approximately to a flow rate of acetylene just sufficient to react with the nitrogen atoms present, as estimated from the hydrogen cyanide produced in experiments with ethylene under similar conditions (8).

\*\*We are indebted to Mr. A. J. Matuszko for the analyses.

reaction vessel contained 32% nitrogen, while that from the traps contained 16% nitrogen. A hydrogen balance at an acetylene flow rate of  $1.2 \times 10^{-5}$  mole per second indicated that the polymer contained only about 20% of the hydrogen originally present in the acetylene that was converted to polymer. Hence, the ratio of carbon to hydrogen in the polymer was probably about five to one.

The polymer was quite insoluble in the usual organic solvents and in dilute acids and alkalis. When heated to about 900°C. in a stream of nitrogen, no cyanogen was detectable but considerable hydrogen cyanide was formed. The residue, amounting to about 50% of the polymer taken, closely resembled graphite in appearance. It would seem, therefore, that the polymer contained little or no paracyanogen, which should decompose completely to cyanogen at 860°C. (7). On the other hand, it has been found (5) that the pyrolysis of methyl or ethyl cyanide at 865°C. and 675°C. respectively produces hydrogen cyanide and considerable amounts of carbon and tar, but no cyanogen. It is suggested, therefore, that the polymer contained nitrogen in a structure analogous to that of a nitrile, and that the monomer from which the polymer was derived may be regarded as a complex formed by collision of a nitrogen atom\* with the acetylene molecule.

From the known amounts of hydrogen cyanide, cyanogen, and polymer produced, together with the approximate values for the nitrogen content (assumed to be 32%) and the carbon-hydrogen ratio of the polymer, the curve for total nitrogen reacted was obtained (Fig. 1D). The shape of this curve is reasonably explained if all the available acetylene is consumed at acetylene flow rates below about  $0.6 \times 10^{-5}$  mole per sec., while at flow rates above  $1.2 \times 10^{-5}$  mole per sec. all available nitrogen atoms are consumed. The curve for recovery of acetylene (Fig. 1E) is consistent with such an interpretation. In the range of acetylene flow rates between  $0.6 \times 10^{-5}$  and  $1.2 \times 10^{-5}$  mole per sec. recovery of acetylene increased slightly until at the upper limit of the range the recovery increased at approximately the same rate as the flow rate (quite apparent in larger scale plot). These observations would appear to imply incomplete reaction in the range of flow rates cited, whereas the small flame size observed in all the experiments would indicate complete consumption of one or other reactant over the entire range of flow rates used. The apparent contradiction can be resolved if it is assumed that nitrogen atoms may be removed from the gas stream by reaction with polymer on the wall. At sufficiently low acetylene flow rates there would still be enough nitrogen atoms present to react with all the hydrocarbon. At sufficiently high acetylene flow rates, nitrogen atoms would be almost completely consumed before significant reaction with the polymer on the wall could occur. At intermediate flow rates of acetylene, however, the extent to which nitrogen atoms were removed by the wall reaction would depend upon the acetylene flow rate. The variable nitrogen atom concentration then available for reaction with acetylene would make the relation between acetylene flow rate and product formation appear to change gradually from one of complete nitrogen atom consumption to one corresponding to complete hydrocarbon consumption. Such

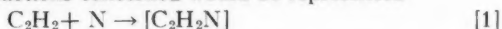
\*If, for example, the  $N_2$  molecule is a reactive species in active nitrogen, formation of such a complex might be represented by  $N_2 + C_2H_2 \rightarrow [C_2H_2N] + N_2$ .

behavior is in marked contrast to the sharply defined conditions for complete hydrocarbon or complete nitrogen atom consumption in the ethylene reaction (3, 8), where virtually no polymer is formed.

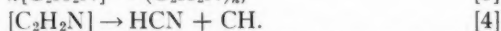
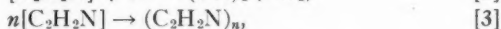
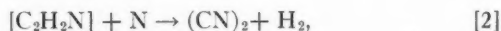
Analyses for methane in the noncondensable products gave quite erratic results, from  $0.3 \times 10^{-6}$  to  $1.8 \times 10^{-6}$  mole per sec. The yields of hydrogen were also erratic, in the range zero to  $2.3 \times 10^{-6}$  mole per sec. However, the total hydrogen in the noncondensable gases gave the regular relation with acetylene flow rate shown in Fig. 1F.

One experiment, at an acetylene flow rate of  $1.08 \times 10^{-5}$  mole per sec., was made with the reaction vessel wrapped in glass wool, so that the temperature indicated by the thermocouple rose to 209°C. Increase in the yields of hydrogen cyanide (10%), cyanogen (35%), and hydrogen (8%), and in consumption of acetylene (25%), were then observed; the polymer yield decreased (25%). No change occurred in the total amount of nitrogen combined in nitrogenous products. The significance of the observed changes is difficult to assess, in view of the uncertainty in the true reaction temperature and in the effect that changes in amount and composition of the polymer on the surface might have on the gas phase reactions. No attempt was made, therefore, to make a more elaborate study of the effect of temperature on the reaction.

A mechanism for the acetylene - nitrogen atom reaction can be formulated in the same way that has been suggested for other reactions of similar type. Collision of a primary acetylene - nitrogen atom complex with a second nitrogen atom would readily account for the cyanogen observed in the products. On the other hand, as indicated previously, the conditions of formation and properties of the polymer obtained are perhaps most satisfactorily accounted for by polymerization of the complex. Decomposition of the complex could, of course, yield hydrogen cyanide. The reactions concerned would be represented

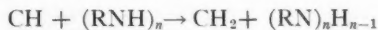


followed by

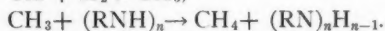
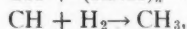


The shape of the cyanogen curve (Fig. 1C) is similar to that observed with ethylene and may be explained in the same way.

The erratic, small amounts of methane found were probably due to reactions of the methyne radical derived from reaction [4]. The lack of reproducibility in the methane values suggests that the polymer might be involved in reactions of the type

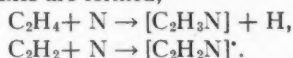


or



The relatively low hydrogen content of the polymer is in accord with the occurrence of such reactions. The regularity of the total hydrogen content of the noncondensable gases (Fig. 1F) compared with the erratic yields of free hydrogen might be accounted for by an erratic consumption of free hydrogen for the production of methane.

A point of interest is the possible difference between the complexes of ethylene and acetylene with nitrogen atoms, such that the acetylene but not the ethylene complex is capable of polymerization. The obvious implication is that the ethylene complex is not a free radical whereas the acetylene complex has free radical character. It is tentatively suggested that the difference may be due to expulsion of a hydrogen atom from the ethylene molecule, but not from the acetylene molecule when the complexes are formed,



Such a concept makes no fundamental alteration necessary in the reaction mechanisms proposed previously for the reactions with olefines or paraffins.

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# ISOLATION OF THE ALDOBIURONIC ACID, 3-(XYLOPYRANOSYL)- $\alpha$ -D-GLUCURONOPYRANOSIDE, FROM WHEAT STRAW HOLOCELLULOSE AND SYNTHESIS OF ITS $\beta$ -ISOMER<sup>1</sup>

By C. T. BISHOP

## ABSTRACT

Extraction of wheat straw holocellulose with 4% potassium hydroxide removed a polysaccharide fraction containing D-xylose (77.2%), L-arabinose (6.9%), and uronic acid anhydride (7.9%). Hydrolysis of this polysaccharide with dilute acid produced a degraded polysaccharide which was precipitated by ethanol after removing free D-xylose from the hydrolyzate by fermentation with *Hansenula suaveolans*. Prolonged hydrolysis of the degraded polysaccharide yielded an aldobionic acid which was shown to be 3-(xylopyranosyl)- $\alpha$ -D-glucuronopyranoside. The  $\beta$ -isomer of this compound was synthesized from D-glucuronic acid and D-xylose and its structure was confirmed by degradative studies after methylation. The enzyme  $\beta$ -glucuronidase did not hydrolyze the  $\alpha$ - or  $\beta$ -isomers of this aldobionic acid.

Uronic acids, when found in polysaccharides, are attached to the main structure of the molecule by a glycosidic bond through their C<sub>1</sub> carbon atoms. Because this glycosidic bond is greatly stabilized by the uronic carboxyl group, hydrolysis of a polysaccharide cleaves the other labile glycosidic bonds first. The acid resistant residue consists of a uronic acid linked glycosidically to one of the carbon atoms of a monosaccharide. An examination of the structure of such compounds, known as aldobionic acids, reveals exactly how two monomeric components of the polysaccharide are joined.

Complex acids consisting of D-xylose units joined to D-glucuronic acid have been found in the hemicelluloses of hardwoods (2, 19), pear cell wall (6), and New Zealand flax (18). However, the position at which the D-glucuronic acid is attached to the D-xylose has never been definitely established. This report discusses the isolation of an aldobionic acid from a wheat straw polysaccharide and the determination of its structure.

Fig. 1 shows the procedure for hydrolysis of the polysaccharide used in this work and isolation of the barium uronates. Analysis of the original polysaccharide showed that it was composed predominantly of D-xylose units together with smaller amounts of L-arabinose and uronic acid anhydride. After hydrolysis and addition of methanol some of the large excess of D-xylose precipitated with the barium uronates. This difficulty was overcome by removing D-xylose from the hydrolyzate by specific fermentation with *Hansenula suaveolans* (24). Preliminary experiments, using known mixtures, showed that D-glucuronic or D-galacturonic acid could be recovered quantitatively when added to fermentations of D-xylose with this yeast.

The uronic acid content of the precipitate, barium salt "A", was 15.1%, and complete methylation, followed by hydrolysis, yielded mono-, di-, and tri-methyl-D-xyloses and a trimethyl hexuronic acid. These results showed

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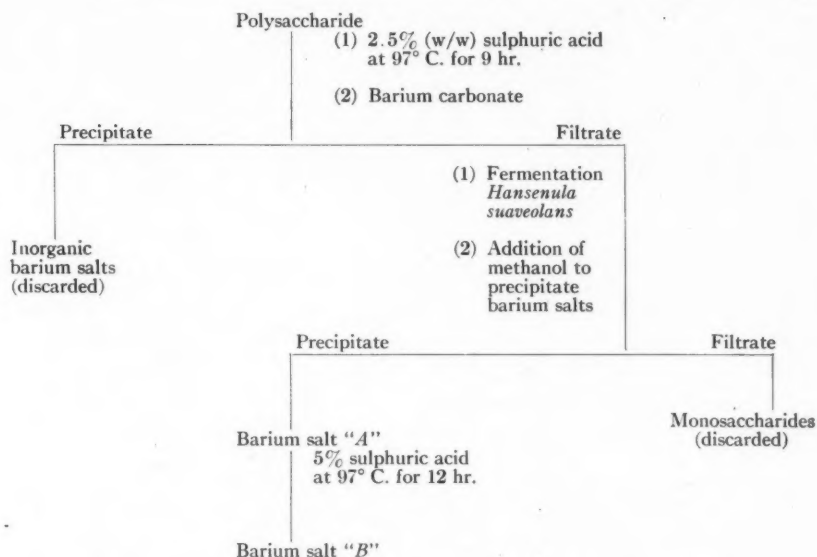


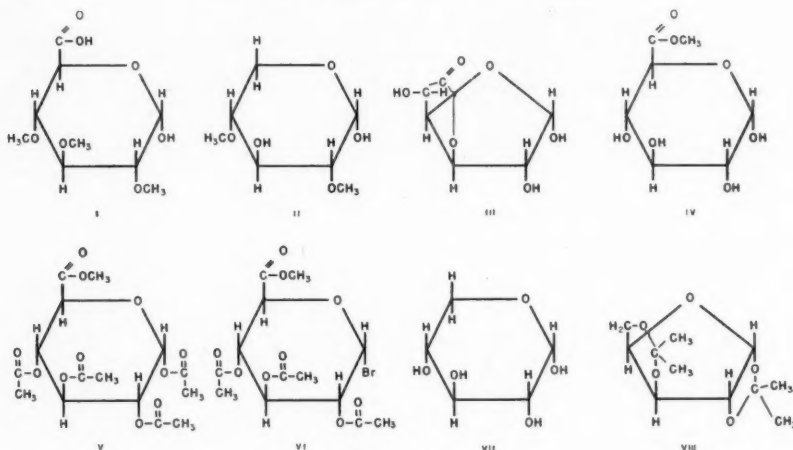
FIG. 1. Hydrolysis of polysaccharide and isolation of the barium uronates.

that barium salt "A" was not the salt of an aldobiuronic acid but was a larger molecule containing at least three D-xylose units together with a uronic acid residue. This compound was similar to the uronic acid complex isolated by McIlroy (18) from New Zealand flax.

Further hydrolysis of barium salt "A" and isolation of the barium uronates gave barium salt "B". Analysis showed that this was the salt of an aldobiuronic acid. In the original polysaccharide and in barium salt "A" the molar ratios of methoxyl to uronic acid anhydride contents approached unity. However, in barium salt "B" the ratio was 0.35 showing that only one-third of the uronic acid anhydride units contained a methyl group. A similar mixture of methylated and nonmethylated uronic acids was found in quince seed mucilage by Renfrew and Cretcher (20). The products of methylation and hydrolysis of barium salt "B" were 2,3,4-trimethyl-D-glucuronic acid (I) and 2,4-dimethyl-D-xylose (II). The identification of these two compounds showed that the aldobiuronic acid was composed of D-glucuronic acid linked glycosidically from its C<sub>1</sub> to the C<sub>3</sub> of a D-xylose unit. The configuration of the glycosidic bond was established by synthesizing the  $\beta$ -isomer and comparing its rotation with that of the naturally occurring aldobiuronic acid.

The aldobiuronic acid, 3-(xylopyranosyl)- $\beta$ -D-glucuronoside was synthesized from D-glucurone and D-xylose. D-glucurone (III) was converted to its methyl ester (IV) which was fully acetylated to the 1,2,3,4-tetraacetyl derivative (V) and then brominated to yield  $\alpha$ -bromo-2,3,4-triacetyl-D-glucuronic acid methyl ester (VI). D-xylose (VII) was converted to 1,2-3,5-diacetone-D-xylofuranose (VIII) from which 1,2-monoacetone-D-xylofuranose (IX) was obtained by





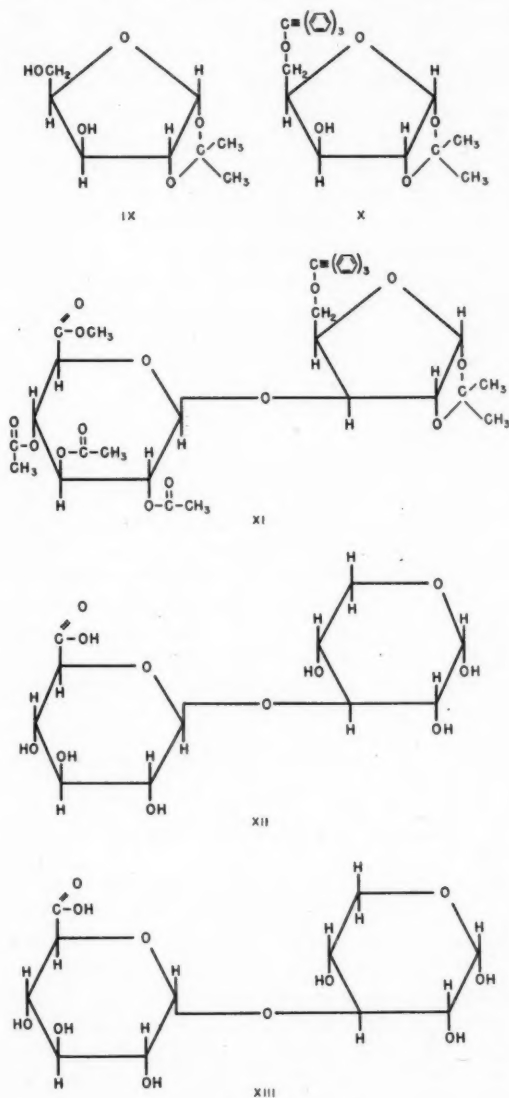
partial hydrolysis. The primary hydroxyl group at  $C_6$  was then blocked by condensation with triphenylchloromethane yielding 1,2-monoacetone-5-trityl-D-xylofuranose (X) in which only the  $C_3$  hydroxyl was free to react. Condensation with the  $\alpha$ -bromo-2,3,4-triacetyl-D-glucuronic acid methyl ester (VI) in the presence of silver oxide formed the glycosidic bond at  $C_3$  of the D-xylose. The condensation product (XI) was freed of its trityl and acetone groups by acid hydrolysis. Alkali saponification removed the methyl ester and acetyl groups to yield the aldobiuronic acid 3-(xylopyranosyl)- $\beta$ -D-glucuronoside (XII). The structure of this compound was confirmed by the same methylation techniques used on the natural aldobiuronic acid. The products of hydrolysis after complete methylation were the same; i.e. 2,3,4-trimethyl-D-glucuronic acid and 2,4-dimethyl-D-xylose.

The specific rotations of the natural and synthetic aldobiuronic acids were not the same. The unsubstituted products were not strictly comparable because approximately one-third of the natural aldobiuronic acid contained a methoxyl group. However, a comparison could be based on rotations of the fully methylated aldobiuronic acids.

	Natural aldobiuronic acid	Synthetic aldobiuronic acid
Unsubstituted	$[\alpha]_D^{27} +18.5$ ( $c = 3.28$ in water)	$[\alpha]_D^{27} -3.97$ ( $c = 1.76$ in water)
Methylated	$[\alpha]_D^{28} +12.4$ ( $c = 1.67$ in chloroform)	$[\alpha]_D^{28} -4.79$ ( $c = 2.25$ in chloroform)

Because in both aldobiuronic acids the D-glucuronic acid was joined glycosidically to  $C_3$  of the D-xylose, the differences in specific rotations could be caused only by a difference in configuration of the glycosidic link. Because  $\alpha$ -bromo-2,3,4-triacetyl-D-glucuronic acid methyl ester (VI), when condensed with alcohols in the presence of silver oxide, yields only  $\beta$ -glucuronides (15), the glycosidic bond in the synthetic aldobiuronic acid must possess the  $\beta$ -con-





figuration. Therefore, the natural aldobiuronic acid (having a different specific rotation) must contain an  $\alpha$ -glycosidic bond. This conclusion is also in agreement with Hudson's rules of nomenclature (13) where the more dextrorotatory member of an  $\alpha$ - $\beta$  pair in the D-series is designated as  $\alpha$ . The aldobiuronic acid isolated from the wheat straw polysaccharide is therefore 3-(xylopyranosyl)- $\alpha$ -D-glucuronoside (XIII).

In aldobiuronic acids the carboxyl group, while greatly stabilizing the glycosidic bond, is itself easily removed by hot mineral acids. Consequently hydrolysis of these compounds by chemical means is always attended by considerable decomposition and poor yields. These difficulties prompted an investigation of the action of the enzyme  $\beta$ -glucuronidase on the two aldobiuronic acids prepared in this work.  $\beta$ -glucuronidase will liberate D-glucuronic acid from its  $\beta$ -glycosidic union with such compounds as phenolphthalein and sterols (16). However, to the author's knowledge hydrolysis of aldobiuronic acids by this enzyme has never been attempted. As reported in the experimental section no activity could be detected. Levvy (17) has reported that this enzyme is strongly inhibited by compounds having structural similarities to D-glucuronic acid. It is possible therefore that the enzyme was inhibited by the D-xylose portion of the aldobiuronic acid.

#### EXPERIMENTAL

##### *Isolation of Polysaccharide*

The polysaccharide was extracted by stirring wheat straw holocellulose (1) in 4% aqueous potassium hydroxide (solid:liquid ratio of 1:30) under an atmosphere of nitrogen for 24 hr. The extract was neutralized with acetic acid and evaporated, under diminished pressure, to  $\frac{1}{4}$  its volume. Addition of four volumes of 95% ethanol precipitated the polysaccharide as white flocs. The precipitate was suspended in water and dialyzed against running distilled water for 48 hr. After reprecipitation with 95% ethanol and drying through ethanol and ether, the polysaccharide was obtained as a white powder in 22% yield based on the ash-free, moisture-free weight of holocellulose.

##### *Analysis of Polysaccharide*

The polysaccharide was analyzed with the results recorded in Table I.

TABLE I  
ANALYSIS OF POLYSACCHARIDE\*

Ash	3.0
Methoxyl	1.6
Uronic acid anhydride ( $\text{CO}_2 \times 4$ )	7.9
D-Xylose	77.2
L-Arabinose	6.9
D-Glucose	Trace
D-Galactose	Trace
Methoxyl/uronic acid anhydride	1.1**

\*All values are in per cent by weight on an ash-free basis.

\*\*Molar ratio.

Ash was determined by incineration at 550° C. and methoxyl by the procedure described by Clarke (7). Uronic acid anhydride was determined by the method of Tracy (23) and individual sugars, liberated by acid hydrolysis to constant reducing power, were determined qualitatively and quantitatively by a combination of chromatographic techniques previously described (4).

##### *Hydrolysis of Polysaccharide*

The polysaccharide (50 gm.) was heated at 97° C. with 2.5% (w/w) sul-

phuric acid (1700 ml.). Hydrolysis was followed by measuring the reducing power of the solution which reached a constant value after nine hours. The hydrolyzate was treated with solid barium carbonate in the usual manner to give a neutral, straw-colored solution (3100 ml.).

#### *Fermentation of D-Xylose*

The neutral hydrolyzate was passed through sterilized Seitz filters, divided into 10 equal portions in Fernbach flasks, and inoculated with *Hansenula suaveolans* (N.R.R.L. No. 838). Yeast extract (0.5%) was added and the flasks were shaken (96 strokes per minute) for 48 hr. at 30° C. Yeast cells were removed by centrifuging and the centrifugate was examined by paper chromatography, using a solvent system of ethyl acetate - pyridine - water (14). Only a trace of D-xylose was found compared with the large quantities present before fermentation. The fermented liquor was acidified (pH 4) with sulphuric acid and proteinaceous material was removed by precipitation with ethanol.

#### *Isolation of Barium Salt "A"*

Ethanol was removed from the protein-free fermentation liquor by azeotropic distillation with water under diminished pressure. The aqueous residue was made alkaline (pH 10) with saturated barium hydroxide and the mixture stirred vigorously for one and one-half hours at 80° C. in an atmosphere of nitrogen. After neutralizing the mixture with carbon dioxide, insoluble barium salts were removed by centrifuging and were washed three times with hot water. Combined washings and centrifugate were freeze-dried and the residue was taken up in water (70 ml.). When this solution was poured into methanol (500 ml.) the water-soluble barium salt "A" was precipitated. Drying through ethanol, ether, and finally over paraffin and anhydrous calcium chloride at 0.1 mm. pressure yielded barium salt "A" as a white powder (5.8 gm.). This material contained 3.0% methoxyl and 15.1% uronic acid anhydride, a molar ratio of methoxyl/uronic acid anhydride of 1.1. Theoretical values for these groups in the barium salt of a monomethyl aldobiuronic acid are 7.6% and 43.2%.

A portion of barium salt "A" (0.5 gm.) was dissolved in dimethyl sulphate (37.5 ml.) and 30% aqueous sodium hydroxide (90 ml.) was added, drop by drop, while the solution was being stirred in an atmosphere of nitrogen. The mixture was stirred at room temperature for 16 hr., then evaporated to 25 ml. under diminished pressure. Sodium hydroxide (90 ml. of 30%) was added and a second methylation was carried out by the dropwise addition of dimethyl sulphate (37.5 ml.). The reaction was worked up as before and a third methylation was carried out using the same quantities of reagents. The mixture was neutralized with 10% sulphuric acid, care being taken to avoid an excess, and sodium sulphate was removed by centrifuging. The centrifugate was extracted exhaustively with chloroform and, after drying over anhydrous sodium sulphate, the extract was evaporated to a syrup (0.266 gm.). This syrup was dissolved in methyl iodide (15 ml.) and heated under reflux. Silver oxide (12.2 gm.) was added in 1/10th portions every half hour, the mixture being continuously stirred. Silver salts were removed by filtration and washed

with hot methanol. Washings and filtrate were combined and evaporated to a syrup which was methylated three more times with methyl iodide and silver oxide. The product (0.176 gm.) had a methoxyl content of 37.0%, unchanged by further methylations. Theoretical methoxyl content for a fully methylated aldobiuronic acid is 51.2%.

The methylated syrup was hydrolyzed by heating under reflux with 7% methanolic hydrogen chloride (10 ml.) for 16 hr. Water (10 ml.) was added and hydrolysis continued at 97° C. for 10 hr. The hydrolyzate was worked up with silver carbonate and hydrogen sulphide in the usual manner and the final filtrate evaporated to 5 ml. Examination of this solution by paper chromatography, using a solvent system of butanol: ethanol: water: ammonia in ratios of 40:10:50:1 (5), indicated the presence of trimethyl uronic acid, mono-, di-, and tri-methyl-D-xylose units.

#### *Isolation of Barium Salt "B"*

Hydrolysis of the polysaccharide was repeated to obtain more barium salt "A" which was combined with the first lot to give a total of 10.2 gm. This salt was hydrolyzed by heating at 97° C. for 12 hr. with 5% (w/w) sulphuric acid (70 ml.). The hydrolyzate was worked up with barium carbonate as before and barium salt "B" was obtained from the neutral hydrolyzate by precipitation with ethanol. Barium content of barium salt "B" was 29.0% but fractionation from water by the gradual addition of 95% ethanol yielded a main fraction having a barium content of 18.1%; calculated for the salt of an aldobiuronic acid, barium = 17.9%. Barium was removed on a column of Nalcite HCR leaving the aldobiuronic acid as a reducing syrup (1.31 gm.) which could not be crystallized. Equivalent weight by titration with 0.107 *N* sodium hydroxide = 305, calculated equivalent weight = 325.  $[\alpha]_D^{25} = +18.5$  ( $c = 3.28$  in water). Methoxyl = 3.23%, calculated for a monomethyl aldobiuronic acid, methoxyl = 9.1%. Methoxyl/uronic acid anhydride = 0.35 (molar ratio).

#### *Methylation of Aldobiuronic Acid*

The syrupy aldobiuronic acid (1.3 gm.) was dissolved in water (10 ml.) and 1 *N* thallos hydroxide (20 ml.) was added to the solution. The mixture was evaporated to dryness at 40° C. in a stream of nitrogen and the residue dried for 18 hr. *in vacuo* at 40° C. over anhydrous calcium chloride. The dry solid was powdered (150 mesh) and boiled under reflux with methyl iodide for 58 hr. Thallos salts were removed by centrifuging and extracted three times with boiling absolute ethanol. The extracts and centrifugate were combined and evaporated to dryness under diminished pressure leaving a brown syrup (0.587 gm.) with methoxyl = 30.0%. This syrup was dissolved in methyl iodide (50 ml.) and methylated by the addition of silver oxide (4.0 gm.) in 1/10th portions every half hour. The mixture was stirred and boiled under reflux throughout the reaction. The methylated product was recovered by evaporation of the filtrate and washings after filtration of the silver salts. Three more methylations by the same method gave a product having a constant methoxyl content of 39.1%. This product was dissolved in

30% sodium hydroxide (30 ml.) and the solution was stirred under nitrogen during the dropwise addition of dimethyl sulphate (15 ml.). The reaction was neutralized with 10% sulphuric acid and exhaustively extracted with chloroform. After the extract had been dried over anhydrous sodium sulphate and evaporated to dryness, a syrup was obtained (0.113 gm.) having methoxyl = 43.4%, unchanged by further methylations, and  $[\alpha]_D^{25} = +12.4$  ( $c = 2.25$  in chloroform). Calculated for a fully methylated, nonesterified aldobiuronic acid, methoxyl = 45.4%.

#### *Hydrolysis of Methylated Aldobiuronic Acid*

The methylated aldobiuronic acid was heated under reflux for 16 hr. with 8% methanolic hydrogen chloride (10 ml.). Water (10 ml.) was added and heating was continued for a further nine hours at 97° C. The hydrolyzate was worked up in the usual manner using silver carbonate and hydrogen sulphide. The product was a reducing syrup that was examined by paper chromatography using a solvent system of butanol: ethanol: water: ammonia (5). Only trimethyl uronic acid and a dimethyl-D-xylose could be detected.

#### *Separation of Trimethyl Uronic Acid and Dimethyl-D-xylose*

The reducing syrup, obtained from the hydrolysis just described, was placed on a column (1.6  $\times$  20 cm.) of powdered cellulose (11). A mixture (40:60 v/v) of *n*-butanol and 80–100° C. petroleum ether was percolated through the column and the eluate was collected in 5 gm. fractions. Fractions 3–5 inclusive gave positive Molisch tests and, when examined by paper chromatography as before, were found to contain only a dimethyl-D-xylose. The trimethyl uronic acid was washed from the column with water.

#### *Identification of 2,4-Dimethyl-D-xylose*

Fractions containing dimethyl-D-xylose were evaporated to dryness, leaving a syrup (55 mgm.) with methoxyl = 34.0, 33.7%; calculated = 34.5%. After the syrup had been seeded with a single crystal of 2,4-dimethyl-D-xylose and cooled at 5° C. for a week, a trace of crystal growth was noticed. Because the quantity of crystalline material was too small for isolation, the anilide derivative was made. The dimethyl-D-xylose was boiled under reflux for two hours in a solution of absolute ethanol (5 ml.) containing aniline (1.3 ml.). Ethanol was removed by evaporation under diminished pressure and after the residue had been cooled at 5° C. for a week the crystalline anilide was removed by trituration with cold ether. This compound was recrystallized from ethyl acetate to a constant m.p. of 168–170° C., identical with an authentic sample of the anilide of 2,4-dimethyl-D-xylose.

#### *Identification of 2,3,4-Trimethyl-D-glucuronic Acid*

The product from the cellulose column was boiled under reflux for eight hours with 5% methanolic hydrogen chloride (10 ml.) to form the methyl ester-methyl glycoside. This compound was a yellow syrup (40.2 mgm.) having a methoxyl content of 56.9, 57.2% and  $[\alpha]_D^{25} = +83.2^\circ$  ( $c = 2.01$  in water). Calculated methoxyl = 58.7% and reported  $[\alpha]_D^{25} = +85^\circ$ . The syrup was dissolved in saturated ammoniacal methanol (20 ml.) at 0° C. and

allowed to stand at room temperature for 48 hr. Evaporation of the solvent under diminished pressure left a semicrystalline residue. Recrystallization from ethanol - petroleum ether yielded the crystalline amide of 2,3,4-trimethyl  $\alpha$ -methyl glucuronoside, m.p. and mixed m.p. 182-183° C.

*Preparation of 1,2-3,5-Diacetone-D-xylofuranose*

Anhydrous D-xylose (25 gm.) was converted to its 1,2-3,5-diacetone derivative by shaking with acetone at room temperature in the presence of sulphuric acid (22). The syrupy product (24.2 gm.) was distilled at 85° C. and 0.3 mm. pressure.  $[\alpha]_D^{27} = +13.8$  ( $c = 4.1$  in water). Reported:  $[\alpha]_D^{18} = +14.0$ .

*Preparation of 1,2-Monoacetone-D-xylofuranose*

1,2-Monoacetone-D-xylofuranose was prepared from 1,2-3,5-diacetone-D-xylofuranose by hydrolysis with 0.2% aqueous hydrochloric acid (10). The product (20.0 gm.) was a syrup.  $[\alpha]_D^{27} = -18.6$  ( $c = 6.24$  in water). Reported:  $[\alpha]_D^{20} = -19.0$  ( $c = 3.0$  in water).

*Preparation of 1,2-Monoacetone-5-trityl-D-xylofuranose*

1,2-Monoacetone-D-xylofuranose was condensed with triphenylchloromethane under conditions described by Levene and Raymond (15). The product (32 gm.) was crystallized from petroleum ether (b.p. 60-90° C.) containing 3% ethyl acetate to a constant m.p. of 118-120° C.;  $[\alpha]_D^{27} = +10.2$  ( $c = 5.49$  in chloroform). Reported: m.p. = 118-119° C.,  $[\alpha]_D^{25} = +10.5$ .

*Preparation of D-Glucuronic Acid Methyl Ester*

This compound was prepared by boiling D-glucurone in anhydrous methanol under reflux (9). The product (32.3 gm.) was not purified but was immediately acetylated by acetic anhydride and pyridine at room temperature.

*Preparation of  $\alpha$ -1,2,3,4-Tetraacetyl-D-glucuronic Acid Methyl Ester*

The mixture of  $\alpha$ - and  $\beta$ -tetraacetyl derivatives from the acetylation was isomerized by treatment with sulphuric acid in a mixture of acetic anhydride and acetic acid (3, p. 490). Recrystallization from ethanol (8) yielded  $\alpha$ -1,2,3,4-tetraacetyl-D-glucuronic acid methyl ester (30.0 gm.); m.p. 110-111° C.,  $[\alpha]_D^{20} = +92.1$  ( $c = 4.58$  in chloroform). Reported: m.p. 111-112° C.,  $[\alpha]_D^{24} = +98.0$ .

*Preparation of  $\alpha$ -Bromo-2,3,4-triacetyl-D-glucuronic Acid Methyl Ester*

$\alpha$ -1,2,3,4-Tetraacetyl-D-glucuronic acid methyl ester (15 gm.) was brominated by hydrogen bromide in acetic anhydride (3, p. 500). The  $\alpha$ -bromo-2,3,4-triacetyl-D-glucuronic acid methyl ester (12.5 gm.) was recrystallized from ether to constant melting point and rotation; m.p. 103-105° C.,  $[\alpha]_D^{20} = +196.5$  ( $c = 0.61$  in chloroform). Reported: m.p. 104-105° C.,  $[\alpha]_D^{25} = +198.0$  ( $c = 0.05$  in chloroform).

*Condensation of  $\alpha$ -Bromo-2,3,4-triacetyl-D-glucuronic Acid Methyl Ester with 1,2-Monoacetone-5-trityl-D-xylofuranose*

$\alpha$ -Bromo-2,3,4-triacetyl-D-glucuronic acid methyl ester (12.5 gm.) was condensed with 1,2-monoacetone-5-trityl-D-xylofuranose (13.6 gm.) in ether



solution, in the presence of silver oxide (11.0 gm.) (12). The reaction product (7.2 gm.) was isolated as sticky crystals from ether. The decomposition point of 103–113° C. was not changed by recrystallization or fractional crystallization.  $[\alpha]_D^{27} = +94.5$  ( $c = 1.18$  in chloroform); saponification equivalent (21, p. 118) = 190, 188; trityl (3, p. 512) = 35.4, 35.3%. Calculated: saponification equivalent = 185, trityl = 35.2%.

#### *Removal of Trityl and Acetone Groups*

The trityl group was removed from the condensation product (7.0 gm.) in chloroform (150 ml.) by saturating the solution with dry hydrogen chloride and allowing the reaction to proceed to constant rotation at room temperature (3, p. 512).

To remove the acetone group, the chloroform solution was evaporated, under diminished pressure, to a syrup. Ethanol (50 ml.) and 3% sulphuric acid (50 ml.) were added and the solution was heated at 97° C. to constant rotation.

#### *Removal of Acetyl and Ester Groups*

The alcoholic hydrolyzate from the removal of the acetone group was freed of ethanol by azeotropic distillation with water. The acidic, aqueous solution was made alkaline (pH 10) with saturated, aqueous barium hydroxide and the mixture was stirred at 70° C. under nitrogen for three hours. Saponification was then allowed to continue at room temperature for 18 hr. After neutralization with carbon dioxide all water-insoluble material was removed by filtration and washed three times with hot water. Washings and filtrate were combined, evaporated under diminished pressure to 75 ml., and poured into 600 ml. of methanol. The precipitated barium salt was removed by centrifuging and was dried through ethanol, ether, and finally over phosphoric anhydride at 0.1 mm. This barium salt was fractionally precipitated from water by the gradual addition of ethanol to yield a main fraction with barium = 18.2%, calculated barium = 17.9%. Barium was removed by percolating an aqueous solution of the salt through a column of Nalcite HCR. The eluate from the column was evaporated to dryness yielding the free aldobiuronic acid as a syrup (1.0 gm.),  $[\alpha]_D^{27} = -3.97$  ( $c = 1.76$  in water).

#### *Methylation of Synthetic Aldobiuronic Acid*

The synthetic aldobiuronic acid was methylated in exactly the same way as already described for the natural product. The methylated product (0.113 gm.) had a constant methoxyl content of 43.6% and  $[\alpha]_D^{28} = 4.79$  ( $c = 1.67$  in chloroform). Calculated methoxyl = 45.4%. Hydrolysis of this methylated aldobiuronic acid and separation of the two components were done as described before. The dimethyl-D-xylose (45 mgm.) was crystallized and gave an X-ray diffraction pattern identical with that given by an authentic sample of 2,4-dimethyl-D-xylose. The anilide, prepared as before, had a m.p. 168–170° C.; reported m.p. 170° C. The trimethyl uronic acid (51 mgm.) was identified as 2,3,4-trimethyl-D-glucuronic acid by conversion to the crystalline amide of its  $\alpha$ -methyl glycoside, m.p. and mixed m.p. 182–183° C.

*Attempted Enzymatic Hydrolyses of Natural and Synthetic Aldobiuronic Acids*

To each of the aldobiuronic acids (0.5 ml. of a 2.6% solution in water) was added 5 ml. of 0.01 *N* acetate buffer (pH 4.5). These solutions were inoculated with 0.5 ml. of a 1% solution of  $\beta$ -glucuronidase, a commercial preparation (Sigma) of bacterial origin. The solutions were then incubated at 38° C. and samples were removed for examination by paper chromatography at 2, 4, 6, 8, 10, 12, and 24 hr. Blanks containing buffer-enzyme and buffer-aldobiuronic acid were also run. No free D-xylose or D-glucuronic acid could be found on the chromatograms although D-xylose, dissolved in acetate buffer at comparable concentrations, was easily detected by this method.

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## MICRODETERMINATION OF COBALT COLORIMETRICALLY AS THE 1-NITROSO-2-NAPHTHOL COMPLEX<sup>1</sup>

BY W. E. NICHOL

### ABSTRACT

An advantage of determining cobalt with 1-nitroso-2-naphthol is that the reagent is relatively stable and forms a very stable cobalt complex. Also this cobalt complex has maximum absorption in the visible spectrum which allows determination visually or with ordinary colorimeters.

The procedure is based on decreasing the iron interference by the removal of part of the iron as phosphate and on a simple purification of the carbon tetrachloride solution of the 1-nitroso-2-naphthol cobalt complex which eliminates traces of palladium, iron, etc.

The method has the same order of accuracy as the *o*-nitrosocresol method but preparation of reagents and the analysis in general require less time.

### INTRODUCTION

Colorimetric determinations of traces of cobalt generally require the elimination of interfering elements. Dithizone extraction (3, 5) of cobalt is often used for separation from iron and from those elements which do not form dithizonates. Interfering elements extracted as dithizonates are then eliminated by other procedures and cobalt is determined by some colorimetric method.

In the method described below the cobalt complex with 1-nitroso-2-naphthol is formed in a pH range where there is a minimum of interference from other metals. It is extracted in carbon tetrachloride. After a rapid purification of this extract, the cobalt in it is determined directly by measuring the intensity of the color of the cobalt complex.

Experiments with pure chemicals showed that ferrous iron interfered through competition for the reagent. The precipitate of ferric phosphate which forms in digests of biological materials at a pH of about 4 also interfered. For general use of the method, elimination of iron interference was desirable.

Willard and Diehl (8) state that the precipitation of iron and some other metals as phosphate is complete at pH 5.0–5.4 in dilute acetic acid – acetate solution but that cobalt forms a double ammonium phosphate which is insufficiently soluble to make possible a separation unless it is present in very small amounts.

North and Wells (6) separated cobalt from iron satisfactorily by precipitating ferric phosphate at pH 3.5. A low pH and the presence of acetic acid tend to keep cobalt in solution (9). It was found that the bulk of the ferric phosphate resulting from constituents of plant ash could be precipitated at an even lower pH—about 2.5—by the method of Willard and his co-workers of gradually and uniformly raising the pH of the solution by the hydrolysis of urea. The precipitate was white and dense. Recoveries of cobalt when using this procedure were good.

In the purification of an extract of the same cobalt complex Paulais (7) removed excess 1-nitroso-2-naphthol by washing with alkali. The rest of his purifi-

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cation process was longer and dissimilar to that outlined below. Boyland (2), using 2-nitroso-1-naphthol, employed acid, alkali, and alcoholic alkali washes. Because of the lessened iron interference and the use of a more selective pH range for the precipitation of cobalt, the purification in this method is shorter than in his.

#### METHOD

##### *Reagents*

(1) *1-Nitroso-2-naphthol*—Weigh 0.5 gm. of 1-nitroso-2-naphthol recrystallized from hot water. Dissolve in and make to 1 liter with cold *N*/10 sodium hydroxide (1).

(2) *Sodium acetate solution*—Dissolve 100 gm. sodium acetate trihydrate in water and dilute to 200 ml.

(3) *Ethyl alcohol - sodium hydroxide wash*—One volume of 95% alcohol plus one volume *N*/10 sodium hydroxide (2).

(4) *Hydrochloric acid*—Redistill from Pyrex.

##### *Procedure*

Weigh into a platinum dish a sample containing 0.2 to 6.0  $\gamma$  cobalt. Ignite in a muffle furnace,\* remove silica as fluoride, and prepare a solution according to the Association of Official Agricultural Chemists method (4).

Place the solution, or a suitable aliquot thereof, in a tall 300 ml. beaker. Make to a volume of about 100 ml., add a few drops of 3% hydrogen peroxide. Heat to boiling and add 5 ml. of 50% sodium acetate solution. If any cloudiness develops add a few drops of hydrochloric acid to clear. To the boiling solution add 10 ml. of a freshly prepared 30% solution of urea and continue boiling. A noticeable cloud generally forms at a pH of about 2.5. Continue the hydrolysis until the pH has risen to 3.5 to 3.9 (pH test paper) and filter off the precipitate immediately. Use a Whatman No. 42 paper and collect the filtrate in a wide-stemmed 125 ml. separatory funnel. To the warm filtrate add 5 ml. of 1-nitroso-2-naphthol reagent. Allow to stand for one hour or more. Extract with a constant quantity of carbon tetrachloride by one of the two procedures described below.

Solutions of plant material when prepared as directed generally form no visible precipitate when treated with 1-nitroso-2-naphthol at a pH of 3.5 to 3.9. In this case one extraction with 30 ml. of carbon tetrachloride is satisfactory. Purify the carbon tetrachloride extract by shaking with the quantities of acid, alkali, and water listed below. With intense colors, read directly. For less intense color, evaporate on the water bath and make to a suitable volume.

A slight precipitate is sometimes present. This may contain complexes of copper, iron, or palladium. Extract with one 10 ml. and four 5 ml. portions of carbon tetrachloride. Flush the film which gathers at the interface after each shaking into the hydrochloric acid funnel, using a small portion of the aqueous phase each time for this purpose. The hydrochloric acid is diluted in this way to a concentration of about 2 + 1. Wash each of the five extracts by shaking successively in each of six separatory funnels containing the following:

\*Treat liver samples (30 gm. wet weight) with 1 ml. concentrated sulphuric acid before ignition.

- No. 1 10 ml. concentrated hydrochloric acid,
- No. 2 20 ml. water,
- No. 3 10 ml. of ethanol - sodium hydroxide reagent,
- No. 4 10 ml. of ethanol - sodium hydroxide reagent,
- No. 5 20 ml. water,
- No. 6 20 ml. water.

Shaking should be vigorous but need not exceed one-half minute by which time the carbon tetrachloride layer clears. After the first two extracts have passed through funnel No. 4, add the wash from this funnel to that in funnel No. 3 and replace it with fresh alcoholic sodium hydroxide. Avoid transfer of the aqueous phase from any one of these funnels to another. Evaporate the combined carbon tetrachloride extracts in a beaker on a water bath to about 2 ml., transfer the remaining liquid to a small volumetric flask, and rinse several times into the flask with carbon tetrachloride.

For examination on the Beckman DU spectrophotometer, make to a volume of 5 ml. and examine at a wave length of 400 m $\mu$ . For examination on the Klett-Summerson or Evelyn colorimeters, make to a volume of 10 ml. The weight of sample used for these last two colorimeters should be at least twice that required for the spectrophotometer.

#### *Blanks*

Run a blank determination covering the entire procedure including the ashing. Run a second blank including that part of the procedure following the elimination of silica. These blanks with reagent-grade chemicals have been found to be the same. If there is a difference, calculate this difference as cobalt and subtract it from the cobalt found.

#### *Preparation of Standard Curves*

To 100 ml. warm water add the quantities of reagents specified in that part of the procedure following the elimination of silica and known amounts of cobalt, based on a standard cobalt solution containing 0.5  $\gamma$  of cobalt per ml. as prepared in (4). Carry through as in the procedure.

#### APPLICATION OF THE METHOD

The method was designed for the analysis of pastures and grains and the livers of the animals feeding on these materials; but it has also been adapted for the analysis of soils. Plant materials so far examined contained less than 0.05% iron and in general ran below 0.01%. Many analyses indicate that Canadian crops contain in general 0.1 to 0.4% phosphorus. Some special crops such as spinach are higher in both iron and phosphorus. In sheep livers 0.04% of iron and 1.3% of phosphorus (DM) may be expected. Cultivated Canadian soils contain in general 0.05 to 0.13% phosphorus and 0.7 to 6.0% iron. Available information indicates that in plants there is about 1 part of cobalt to 500 parts of iron, in livers 1 part to 1000 parts or more of iron, and in soils 1 part to 5000 parts of iron. More interference might be expected from iron in livers and soils than in plants.

Soils are different in that naturally-occurring phosphorus is not nearly sufficient to precipitate the iron. Rather than adding phosphate it has been found desirable to avoid large precipitates by extracting part of the iron from the hydrochloric

acid solution of the ash by an ether extraction before application of the procedure outlined above. Ether extraction is desirable for all materials high in iron.

The one-extraction procedure gives the same standard curve and the same analysis of plant materials as the longer five-extraction procedure but is more dependent upon proper manipulation. The recoveries of added cobalt shown in Table I were obtained by the longer method. The quantities of cobalt shown in

TABLE I

Cobalt added, γ	Total cobalt, γ	Cobalt found, γ	Recovery of added cobalt, %
<i>Extracted linseed meal</i> —Size of sample 2.5 gm. Analysis 0.47 p.p.m.			
0	1.175		
0.25	1.425	1.425	100
0.50	1.675	1.70	105
0.75	1.925	1.91	98
1.0	2.175	2.175	100
1.5	2.675	2.60	95
<i>Oats</i> —Size of sample 8.0 gm. Analysis 0.035 p.p.m.			
0	0.28		
0.25	0.53	0.53	100
0.50	0.78	0.77	98
1.0	1.28	1.26	98
<i>Barley</i> —Size of samples 8.0 and 7.0 gm. Analysis 0.096 p.p.m.			
0	0.77		
0.25	1.02	1.00	92
0.50	1.27	1.26	98
0	0.67		
0.50	1.17	1.10	86
0.75	1.42	1.40	97
1.00	1.67	1.57	90

the first column were added to samples containing the amount of cobalt shown at the top of column 2. The other figures shown in column 2 are the sum of added and naturally-occurring cobalt. The total cobalt recovered is shown in column 3. The per cent recoveries, column 4, are considered satisfactory for this kind of analysis.

Tables II and III show comparative analyses of livers and soils carried out by the 1-nitroso-2-naphthol method and by the *o*-nitrosocresol method which is generally considered the most accurate method available.

TABLE II  
LIVERS

Sample number	1-Nitroso-2-naphthol method cobalt, p.p.m.*	<i>o</i> -Nitrosocresol method cobalt, p.p.m.*
1	0.114	0.112
2	0.046	0.046
3	0.106	0.099
4	0.087	0.086
5	0.041	0.055

\* Fresh weight basis.



TABLE III  
SOILS

Sample number	1-Nitroso-2-naphthol method cobalt, p.p.m.*	<i>o</i> -Nitrosocresol method cobalt, p.p.m.*
1	8.8	9.2
2	16.0	18.0
3	2.8	2.8

\*Air-dry basis.

## DISCUSSION

The procedure outlined eliminates effective competition of iron for the reagent. Variations in materials and ashing procedures may lead to the persistence of ferrous iron in the hydrochloric acid solution of the ash. A treatment with peroxide, more thorough than that specified, is then necessary but a survival of peroxide should be avoided because of its action on the cobalt-1-nitroso-2-naphthol complex. Ferrous iron forms a green complex with 1-nitroso-2-naphthol. The presence of a green color or precipitate may indicate excessive competition and incomplete reaction of the cobalt.

Removal of iron by this method compares favorably in time with its removal by dithizone procedures.

The procedure for purifying the extract of the cobalt complex also compares favorably in time and effectiveness with purification by other methods to which reference has been made.

In comparison with the *o*-nitrosocresol method (4), this method has approximately the same degree of accuracy and is more rapid, particularly where occasional samples are to be tested. All reagents can be prepared in about one hour. The 1-nitroso-2-naphthol reagent is relatively stable. The cobalt complex has maximum absorption in the visible spectrum rather than in the near ultraviolet. It is thus adapted for visual estimation and for use with ordinary colorimeters. A further advantage in the analysis of some materials by this method is the elimination of the interference of traces of palladium.

The cobalt complex with 1-nitroso-2-naphthol is stable and there is no need for immediate examination. This gives the 1-nitroso-2-naphthol an advantage over the nitroso-R-salt-bromine method.

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# THE DIFFUSION OF POLYISOBUTYLENE IN DIFFERENT SOLVENTS<sup>1</sup>

By C. LUNER<sup>2</sup> AND C. A. WINKLER

## ABSTRACT

The diffusion coefficient of polyisobutylene in *n*-heptane has been found to decrease at first, then to increase with increasing molecular weight.

## INTRODUCTION

In a previous paper (1) it was shown that in methyl ethyl ketone the diffusion coefficient of polystyrene first decreased, then increased slightly with a further increase in molecular weight.

To determine whether a similar behavior might be observed with some other linear polymer the diffusion coefficient - molecular weight relation of polyisobutylene has been investigated in some different solvents.

## APPARATUS AND MATERIALS

The diffusion cells and experimental technique were the same as those used by Adelstein and Winkler (1).

The following unfractionated polymers were used in the study.

Polymer designation*	Molecular weight**
H-300	1000
4227	3000
4186	6000
4224	6500
4184	13,500
4225	20,400
4092	24,000
4116	32,700
4228	41,750
4223	110,000
4222	185,000
4220	447,000

\* Sample H-300 was obtained through the courtesy of Indopol Ltd., while the remaining polymers were kindly supplied by Polymer Corporation, Sarnia.

\*\* The molecular weight of H-300 was determined by the Rast method (4). Molecular weights of the other polymers were determined by viscosity measurements, using the viscosity - molecular weight equation,  $(\eta) = kM^a$ , where  $k$  and  $a$  have the values  $3.6 \times 10^6$  and 0.64 respectively.

The solvents were obtained from Brickman and Co., Montréal, and a middle fraction taken for use.

To prepare solutions of the high molecular weight polyisobutylene, the polymer was cut into small strips and allowed to soften and swell in the solvents. When the polymer appeared to be soft, the mixture was stirred.

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<sup>2</sup> Holder of Quebec Provincial Scholarship, 1948-52.

The resulting solutions were filtered since they were turbid and contained some insoluble material. Polymer concentrations were determined by evaporation of an aliquot of the solution to constant weight.

Using samples of H-300, the variation in values of the diffusion coefficient determined in different cells was within the limits of reproducibility of the results ( $\pm 8\%$ ).

#### RESULTS AND DISCUSSION

The effect of molecular weight upon the diffusion coefficient was determined in benzene, carbon tetrachloride, and *n*-heptane. The results are plotted in Fig. 1. While the general type of inverse relation predicted by many workers

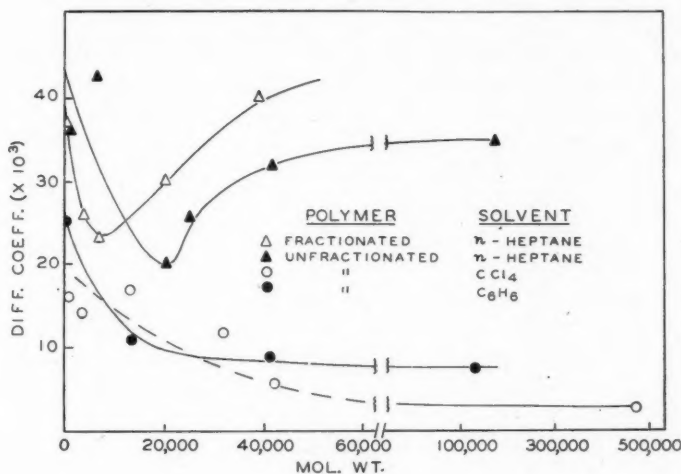


FIG. 1.

was valid for benzene and carbon tetrachloride, the diffusion coefficient-molecular weight relation when *n*-heptane was the solvent was similar to that observed by Adelstein and Winkler for the diffusion of polystyrene in methyl ethyl ketone (1).

To determine whether the distribution of molecular weights in the unfractionated polymers was responsible for the increase in diffusion coefficient with molecular weight, fractions of different molecular weights were obtained from the whole polymer by precipitation with methanol from dilute solutions in benzene. The diffusion coefficients in *n*-heptane are shown in Fig. 1.

Comparison of the behavior of polystyrene and polyisobutylene in different solvents indicates that, whereas viscosity and diffusion data are in agreement as to the relative goodness of solvent for polystyrene, there is a discrepancy between solvent power determined from viscosity and diffusion data for polyisobutylene. According to the present investigation, *n*-heptane is a poorer solvent for polyisobutylene than benzene, whereas viscosity data (2, 3) indicate the reverse to be true. Since the behavior of polyisobutylene in *n*-heptane

would indicate *n*-heptane to be a poor solvent (higher diffusion rate), the increase in diffusion coefficient with increase in molecular weight might be explained in a manner similar to that proposed for the analogous behavior of polystyrene in methyl ethyl ketone (1).

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## SYNTHESIS OF ORGANIC DEUTERIUM COMPOUNDS

### IV. METHYL- $d_3$ BROMIDE<sup>1</sup>

BY B. NOLIN AND L. C. LEITCH

#### ABSTRACT

Silver acetate- $d_3$  and bromine react readily in the dry state in a sealed tube to give methyl- $d_3$  bromide in high yield. This method is more convenient than the reduction of carbon dioxide to deuterated methyl alcohol with lithium aluminum deuteride, followed by the conversion of the alcohol into the halide, or other methods hitherto reported.

#### INTRODUCTION

As potential sources of deuteromethyl groups, methyl- $d_3$  halides are very valuable compounds in deuterium chemistry. Deuterated methyl halide of high isotopic purity was needed in these laboratories for research on reaction mechanisms, free radicals, and infrared absorption spectra of organic molecules.

Two methods of preparation are available in the chemical literature but both are unsatisfactory in some respects. In 1942, Noether (9) reported the synthesis of two deuterated methyl halides from nitromethane according to the reaction scheme I ( $X = \text{Cl, or Br}$ ).



This method is inconvenient owing to its length and the low over-all yield.

Beersmans and Jungers later prepared the methyl- $d_3$  halides (2) via methyl- $d_3$  alcohol (1) according to the reaction scheme II ( $X = \text{Cl, Br, or I}$ ).



The reduction of carbon monoxide was carried out in the presence of a copper-zinc-chromium (49:43:8) catalyst in a specially designed apparatus at 280°C. under a pressure of 3.5 atm. of deuterium gas. The yield of alcohol was very good and the methyl group contained 98 atom % deuterium as determined by infrared absorption. This method, however, requires elaborate apparatus and gives a low daily yield of alcohol (1.5–2.0 ml.). The synthesis of a large amount of alcohol requires many days of continuous operation, while the preparation of a small quantity can hardly justify the use of this process. Moreover, the primary reaction product is the alcohol, whereas in many cases the material required is the halide; the conversion usually gives a yield of 80%.

The reduction of carbon dioxide with lithium aluminum hydride in diethyl carbitol solution was reported to give methanol in 80% yield based on carbon dioxide (11); the yield based on the hydride is however only 40%. Isotopically labeled carbon dioxide was later reduced in a similar way, the yields of methanol and methyl iodide being respectively about 90% and 80% (3, 4); on the basis of the hydride used, they are reduced by half.

In the present work, lithium deuteride was prepared in nearly quantitative

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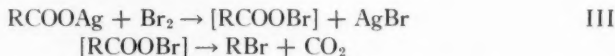
yield from lithium metal and deuterium gas (99.7 atom % deuterium) in a specially designed reactor at 750°C. The finely ground material was reacted with a diethyl ether solution of aluminum chloride, essentially as described for the preparation of lithium aluminum hydride (5), to give lithium aluminum deuteride in 70% yield, based on lithium deuteride. The compound contained 94.4 atom % deuterium as determined by mass analyses of the deuterium gas evolved on pyrolysis (5).

Carbon dioxide was reduced with lithium aluminum deuteride in diethyl carbitol solution essentially as described for the reduction of isotopically labeled carbon dioxide with lithium aluminum hydride (3), except for the use of a smaller excess (1.33 equivalents) of the reducing agent. The crude deuterated methanol was converted into the iodide by means of a Zeisel reaction in an over-all yield of 40%. This method is tedious and somewhat hazardous. The yield is low and the contamination of the final product with about 5% of normal ethyl iodide is inherent to the use of diethyl carbitol as solvent. This contamination can be reduced to a certain extent by the use of tetrahydrofurfuryloxymethyltetrahydropyran (3, 4), but this solvent is not available commercially and does not improve the yield significantly.

The isotopic purity of the deuterated methyl iodide corresponded to 94.3 atom % deuterium, which is in agreement with the figure found for lithium aluminum deuteride. However, this is lower than that of the deuterium gas used in the preparation of lithium deuteride. No sure explanation is offered for this dilution effect, as precautions were taken during the synthetic process to exclude any obvious source of contamination. The lithium metal might have occluded some hydrogen which eventually diluted the deuterium gas.

In a previous paper from these laboratories (10), one of us mentioned the preparation of deuterated methyl bromide in 25% yield from deuterated silver acetate and bromine in carbon tetrachloride solution; the isotopic content amounted to 90 atom % deuterium. The reaction was not further investigated at the time, but it was felt that a procedure might be developed to give satisfactory yields of pure deuterated methyl bromide of high isotopic purity. This paper describes experiments made in this direction.

Silver salts of monobasic carboxylic acids react with two equivalents of bromine as represented in the simplified scheme III (8).



The reaction is usually carried out in carbon tetrachloride solution. According to Hunsdiecker *et al.* (7) however, methyl bromide was prepared in 80% yield in the absence of solvents in a *suitable vessel*, the nature of which was not specified in the patent.

In the present procedure, silver acetate- $d_3$  is treated in the dry state with a slight excess of bromine in the apparatus illustrated in Fig. 1. This tube permits both reagents to be present under reduced pressure in the same reaction vessel so that their contact may be controlled as desired. Features of the decomposition of the intermediate complex  $[\text{RCOOBr}]$  are the low temperature at which it



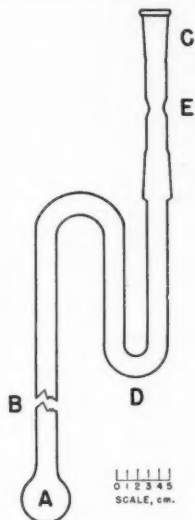


FIG. 1. Apparatus for the reaction of bromine with silver acetate- $d_3$ .

occurs, and the fact that as the chain length of the acid is shortened, the decomposition occurs with increasing violence (12, 13). Under the conditions described in the experimental part, the reaction of bromine with silver acetate- $d_3$  is smooth and complete in about half an hour. The crude material is readily purified and 75–82% yields of methyl- $d_3$  bromide are consistently obtained. Its isotopic purity was 99.3 atom % deuterium, which is assumed to be also the deuterium content of its precursors, namely deuterated silver acetate, acetic acid, and malonic acid.

The latter acid was easily prepared on the one-mole scale by repeated exchange of normal malonic acid with deuterium oxide. By decarboxylation, deuterated acetic acid was obtained in nearly quantitative yield. Deuterated malonic acid could also be prepared by direct synthesis from carbon suboxide and heavy water (6, 14).

#### EXPERIMENTAL

##### *Silver Acetate- $d_3$*

A given weight of deuterated acetic acid is diluted with cold water and the solution neutralized carefully with cold dilute ammonia. On adding silver nitrate deuterated silver acetate is precipitated; it is isolated by filtration, washed with cold water, and dried. The material present in the filtrate is easily recovered by concentration. The yield of the silver salt is practically quantitative. If the silver salt, for some reason, is not free from acetic acid, it is preferably purified by further washing with or crystallization from water, as traces of the acid have been found to reduce the yield of the subsequent preparation.

##### *Methyl- $d_3$ Bromide*

The preparation is carried out in the apparatus illustrated in Fig. 1.

Silver acetate- $d_3$  (0.05 mole, 8.5 gm.) is placed in the 25 ml. flask *A*, which is sealed to an inverted S-shaped tube at *B*, and dried by heating in boiling water under vacuum. Bromine (0.06 mole, 3.1 ml.), dried over phosphorus pentoxide, is distilled from a vacuum line through *C* and frozen at *D* with liquid nitrogen (a dry ice-acetone mixture could likely be used). The reactor is then sealed off at *E*. The bromine is melted by plunging *D* into an acetone bath at room temperature and, by cooling the reaction flask till the bulk of bromine begins to boil, some is frozen above *A*. This portion of bromine is melted and reacted slowly with the silver salt while the tube is being swirled. The process is repeated about ten times and by then the reaction mixture has already yielded some methyl bromide. The reaction is thereafter continued as follows: *A* is cooled up to *B* and some bromine\* is allowed to run down to the reaction flask and frozen above *A*; the more volatile methyl bromide is frozen at *D*, which facilitates the reaction of the bromine frozen above *A*. The process is repeated till all the liquid at *D* is used up. The reaction time, as estimated from 10 runs, varies from 30 to 45 min.\*\* The reaction mixture was generally allowed to stand overnight in the dark.

The reaction tube is cooled at *D*, opened, and attached quickly to the evacuated manifold. The system is completely evacuated and all the volatile material is allowed to distill out. The yellow residue in *A* is heated with a free flame to liberate any remaining methyl bromide from the intermediate complex.

The crude material is passed through a U-tube filled with Ascarite, where carbon dioxide and most of the excess bromine are removed. A similar treatment with Ascarite and Drierite removes the last traces of bromine and any water, the presence of which is however unlikely under the conditions described above. In some runs, a yellow coloration persisted, which disappeared on further treatment with Ascarite. Vapor pressure measurements and mass analyses indicated that the deuterated methyl bromide thus obtained is slightly contaminated with deuterated ethane, acetic acid, and possibly methyl acetate. These by-products are easily removed in the following manner. The material is cooled at  $-78^\circ\text{C}$ . and its vapor is allowed to expand four times into a manifold, the fractions being discarded. Four distillations at  $-78^\circ\text{C}$ . are then carried out from bulb to bulb to remove the less volatile impurities. Yield, 4.0 gm., 81.7%.

The above treatment yields a product of high chemical purity, as judged by its mass spectrum and its vapor pressure at  $0^\circ\text{C}$ ., 681.5 mm. This figure is in agreement with that reported by Beersmans and Jungers (2) for pure methyl bromide of comparable deuterium content. Mass analyses have indicated an isotopic purity of 99.3 atom % deuterium (98.0%  $\text{CD}_3\text{Br}$  and 2.0%  $\text{CD}_2\text{HBr}$ ). This is the equivalent of 2.98 deuterium atoms per methyl group, while the theoretical requirement is 3.

#### ACKNOWLEDGMENT

The authors wish to thank Dr. F. P. Lossing, Miss F. Gauthier, and Miss J. Fuller for the mass analyses.

\*Very small portions of bromine are introduced at first, but larger amounts can be added when the reaction subsides.

\*\*One must not be anxious to bring about the reaction in a shorter time, even when it seems to proceed smoothly. It is preferable to warm up the reaction flask to room temperature before further addition of bromine; this avoids the accumulation of much undecomposed complex in the reaction mixture.

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# PHOTOLYSIS OF ACETONE - HYDROGEN CHLORIDE MIXTURES<sup>1</sup>

BY R. J. CVETANOVIĆ<sup>2</sup> AND E. W. R. STEACIE

## ABSTRACT

The photolysis of acetone - hydrogen chloride mixtures has been investigated at 150° C. and at room temperature. A strong suppression of ethane formation with a corresponding large increase in the formation of methane results from additions of relatively very small amounts of hydrogen chloride to acetone. The importance of the reactions



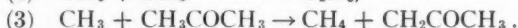
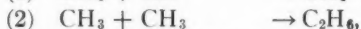
and



has been demonstrated. The collision yield of reaction (1) at 28° C. is  $2 \times 10^{-4}$ , and, therefore, the upper limit for  $E_1$  is 5.1 kcal. per mole. The effects observed at 150° and 28° indicate that, on the assumption of a zero activation energy and a steric factor of unity for combination of methyl radicals,  $E_1 = 2.1 \pm 1$  kcal. per mole, and  $P_1$  is approximately  $7 \times 10^{-3}$ .

## INTRODUCTION

Methyl radicals produced in the photolysis of acetone have been widely used in the investigation of hydrogen abstraction reactions. Recently these investigations have been extended to halide substituted hydrocarbons. Raal and Steacie (12) have determined the activation energies and the steric factors of the reactions of methyl radicals with a number of halogenated methanes in the temperature range 110 to 220° C. The following mechanism of methane and ethane formation is proposed:



For experimental conditions where the concentrations of acetone and RH can be regarded constant, and assuming a time independent concentration of methyl radicals, it can be shown that

$$[1] \quad \frac{k_1}{k_2^{1/2}} = \frac{\frac{R_{\text{CH}_4}}{(R_{\text{C}_2\text{H}_6})^{1/2}} - \frac{k_3}{k_2^{1/2}} [\text{CH}_3\text{COCH}_3]}{[\text{RH}]}$$

Experimental measurements of the rates of formation of methane ( $R_{\text{CH}_4}$ ) and ethane ( $R_{\text{C}_2\text{H}_6}$ ) in the absence and presence of RH are used to evaluate  $k_3/k_2^{1/2}$  and  $k_1/k_2^{1/2}$  respectively. Arrhenius plots of such determinations over a temperature range give the values of  $(E_3 - \frac{1}{2} E_2)$  and  $(E_1 - \frac{1}{2} E_2)$ . Since  $E_2$  is approximately zero, these values are to a good approximation identical with the activation energies of the respective elementary reactions (1) and (3).

For the series of reactions of methyl radicals with  $\text{CH}_3\text{Cl}$ ,  $\text{CH}_2\text{Cl}_2$ , and  $\text{CHCl}_3$ , Raal and Steacie thus find a substantial increase in the ease of removal of hydrogen as the number of chlorine atoms in the molecule increases. This

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<sup>2</sup> National Research Council of Canada Postdoctorate Fellow, 1951-52.

result is not unexpected in view of the other observations on the reactivity of these compounds. The authors have, however, also observed a very pronounced effect of carbon tetrachloride on the rates of formation of methane and ethane in the photolysis of acetone. The effect is analogous to that produced by chloroform. Relatively small amounts of carbon tetrachloride added to acetone suppress very strongly formation of ethane and the formation of methane is correspondingly increased. While in the case of partially halogenated methanes the increased methane formation is easily explained by hydrogen abstraction, the corresponding effect due to additions of carbon tetrachloride is entirely unexpected. The apparent anomaly introduces, therefore, an element of uncertainty into the above results. The authors' suggestion that the observed anomaly may be due to small amounts of hydrogen chloride, present as an impurity or formed during the photolysis, makes it necessary to obtain some quantitative information on the effect produced by additions of varying amounts of hydrogen chloride to acetone.

In a recent paper A. S. Kenyon (9) reports formation of hydrogen chloride as a product in the photolysis of acetone in the presence of *sec*-butyl chloride. At the same time ethane formation is completely suppressed even at 25°. The author ascribes the latter fact to a rapid reaction of methyl radicals with *sec*-butyl chloride. The possibility of an effect of the presence of hydrogen chloride is not considered.

Williams and Ogg (17) have investigated the rate of reaction of methyl radicals, produced by photolysis of methyl iodide, with hydrogen chloride. They find the activation energy of this reaction only about 1.6 kcal. per mole greater than that of the rapidly occurring reaction with hydrogen iodide, although they suggest that in the former case a reaction of "hot" methyl radicals is of importance.

Both in the older and the recent literature a somewhat controversial attitude with respect to the importance of the reaction between methyl radicals and hydrogen chloride is found. At the same time it can be conceived that in the photolysis of acetone in the presence of chlorine containing compounds hydrogen chloride might be formed in the process or could be introduced in varying amounts as an impurity. It was, therefore, considered of interest to investigate separately its effect. A largely qualitative preliminary experiment, conducted in collaboration with Dr. F. A. Raal, showed that the addition of a relatively small amount of hydrogen chloride to acetone photolyzed at 150° suppressed ethane formation almost completely and the amount of methane produced was much increased. A quantitative investigation of this effect necessitated some arrangement for the introduction of known very small quantities of hydrogen chloride into the reaction system. Such an investigation, conducted at 150° and at room temperature, is reported in this paper.

#### EXPERIMENTAL

##### *Apparatus*

The apparatus was of the same general design as used previously by Raal and Steacie (12). Alterations were made: (1) to allow introduction of known

small quantities of hydrogen chloride and to ensure thorough mixing with acetone; (2) to eliminate mercury vapor as much as possible from the reaction cell. The former was achieved by a system of calibrated volumes and by ultimately freezing over the two reactants into a 300 ml. calibrated bulb and, on expansion, allowing sufficient time for interdiffusion; the mixed reagents were subsequently expanded into the reaction cell. The latter was accomplished by measuring the pressures of vapors of the reactants contained in large accessory volumes, which were filled simultaneously with the reaction system. The accessory volumes were subsequently isolated from the reaction system and opened to mercury manometers. A correction for the slight increase in volume (amounting to 1 to 2%) was applied to the measured pressure. Repeated calibration and testing of this arrangement ensured satisfactory accuracy.

The light from a Hanovia S-500 medium pressure arc was roughly collimated by a highly polished aluminum cylinder. For most determinations the light beam was passed through a Corning 9-54 filter, and in a few determinations a Corning 0-53 filter was used instead. In the former case the light beam contained the wave lengths greater than about 2200 Å, in the latter greater than about 2800 Å. The light beam filled the cylindrical silica reaction cell 120 ml. in volume.

The reaction cell was contained in an aluminum block furnace. The heating current was supplied from a Sorensen voltage regulator and the temperature was maintained to within about 1°.

The products were analyzed for methane, ethane, and carbon monoxide. The analysis system was of the conventional type. Carbon monoxide was determined by combustion over copper oxide at 215°. In a number of cases simultaneous determinations were made by mass spectrometer analysis as a check. Ethane was separated at -165°. At that temperature no hydrogen chloride was recovered from acetone, as was evident from determinations where large amounts of hydrogen chloride were added to acetone and the ethane fraction was negligibly small.

#### Materials

Acetone was a Mallinckrodt Analytical Reagent product, refluxed over potassium permanganate, dried over Drierite, and distilled. It was degassed in the usual manner by bulb to bulb distillation *in vacuo*. Hydrogen chloride was a Matheson Company product. It was degassed and further purified by several bulb to bulb distillations *in vacuo* (dry ice - acetone to liquid air). The deuterated acetone (87%  $d_6$ ) was made by Dr. L. C. Leitch in this laboratory.

#### RESULTS

The photolysis of acetone in the presence of hydrogen chloride was investigated at 150° and at room temperature. Most determinations were carried out with a light beam of wave lengths greater than about 2200 Å and only a few determinations were made with wave lengths greater than about 2800 Å. In all cases addition of hydrogen chloride led to an increase in the amount of



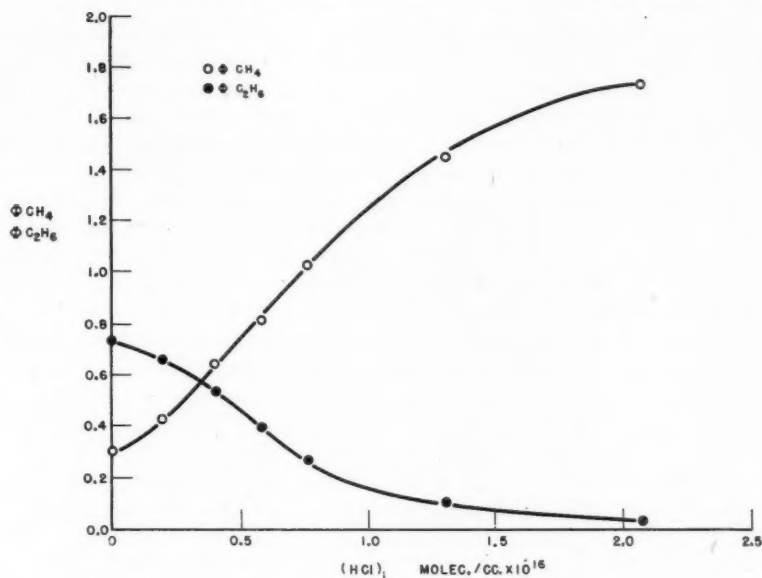


FIG. 1.

methane formed with a corresponding decrease in ethane formation. Fig. 1 shows the quantum yields of methane and ethane at  $150^\circ$  and  $\lambda > 2200 \text{ \AA}$  for varying initial hydrogen chloride concentrations, the other experimental factors being kept approximately constant.

Relatively small amounts of hydrogen chloride (less than 1 mm. added to 100 mm. of acetone) were sufficient to suppress ethane formation almost completely; the formation of methane was correspondingly increased and approached at  $150^\circ$  a value close to twice the amount of carbon monoxide produced. The yield of carbon monoxide was not or possibly was only slightly affected.

It was found convenient to investigate the influence of different experimental conditions by following trends in the values of  $k_1/k_2^{1/2}$  calculated from Equation [1] after substituting in it  $[\text{HCl}]_i$  for  $[\text{RH}]$ . It is, therefore, implicitly assumed that hydrogen chloride concentration does not vary with time and is equal to the concentration of the initially introduced hydrogen chloride ( $\text{HCl}_i$ ). (In the following the symbol  $(k_1/k_2^{1/2})_i$  will denote the values obtained to this approximation.) The reason for this choice may be evident from the following considerations. The amounts of hydrogen chloride added to acetone in these experiments are much too small to account for the increased methane production if it is to be assumed that the additional methane is formed by reaction (1), i.e., hydrogen abstraction from the originally introduced hydrogen chloride. At the same time, as will be seen from the subsequent discussion, there is evidence that reaction (1) is involved in the process. It seems, therefore, to be necessary to assume, as was done previously by Williams and

Ogg (17), that hydrogen chloride is regenerated, in this case by reaction of chlorine atoms with acetone. If the hydrogen chloride regeneration is complete, and since in all experiments large concentrations of acetone are used and the reactions are carried out to small conversions, the concentrations of hydrogen chloride and acetone remain constant. Equation [1] can then be used for evaluation of  $k_1/k_2^{1/2}$ . If the hydrogen chloride regeneration is not complete, the effective mean hydrogen chloride concentration will be smaller than the initial hydrogen chloride concentration and the calculated values of  $k_1/k_2^{1/2}$  will be too small. A criterion of the completeness of the hydrogen chloride regeneration is, therefore, constancy of  $(k_1/k_2^{1/2})_1$  for different experimental conditions.

Typical results of the experiments carried out are presented in Tables I to IV. An attempt was made to investigate trends in the values of  $(k_1/k_2^{1/2})_1$  by varying one experimental factor at a time. This, however, was achieved only approximately. For this reason a graphical representation of the results would show a scattering of points not necessarily representative of the actual experimental error. As a result of the necessity of keeping all experimental factors constant but one, in some of the experiments the amounts of the products are very small and may involve a considerable analytical error. They are believed, however, to indicate correctly the trends in the values and have been, therefore, included in the tables.

Table I shows drifts in the values of  $(k_1/k_2^{1/2})_1$  with increasing initial hydrogen chloride concentrations for the photolysis of acetone at 150°. Acetone pressure was kept at approximately 100 mm. The first five determinations were carried

TABLE I  
PHOTOLYSIS OF ACETONE-HCl MIXTURES AT 150° C.  
Effect of HCl concentration

Run	Temp., ° C.	[HCl] <sub>i</sub> , molec./cc. × 10 <sup>-16</sup>	[Ac], molec./cc. × 10 <sup>-18</sup>	Time, sec.	In molec./cc. sec. × 10 <sup>-13</sup>			$\frac{C_2H_6 + \frac{1}{2} CH_4}{CO}$	$(k_1/k_2^{1/2})_1$ × 10 <sup>9</sup>
					R <sub>CO</sub>	R <sub>CH<sub>4</sub></sub>	R <sub>C<sub>2</sub>H<sub>6</sub></sub>		
Filter 9-54									
23	149.5	....	2.33	1800	6.69	1.71	5.11	0.88	...
22	151.0	0.58	2.29	1800	6.77	5.50	2.66	0.75	1.4
21	151.8	0.76	2.28	1800	7.00	7.19	2.28	0.76	1.9
20	150.4	1.31	2.28	1800	7.34	10.6	0.759	0.82	2.8
19	148.2	2.07	2.32	1800	8.30	14.4	0.251	0.89	4.3
37	151.1	....	2.52	2400	5.92	1.77	4.28	0.87	...
34	152.6	0.19	2.30	2400	6.48	2.78	4.27	0.87	0.9
32	151.4	0.40	2.66	2400	7.17	4.61	3.79	0.85	1.1
31	150.0	0.62	2.39	2400	6.92	5.68	2.80	0.85	1.3
35	147.0	1.50	2.14	2400	5.65	9.34	0.234	0.87	3.9
Filter 0-53									
67	150.6	....	2.28	10800	0.195	0.180	0.054	0.70	...
66	150.6	0.38	2.29	10800	0.199	0.326	0.005	0.84	3.1
65	150.5	3.14	2.31	10800	0.212	0.401	.....	0.96	...

out under conditions of free access of mercury vapor into the reaction cell from the adjoining mercury manometer. In the case of all the remaining determinations reported mercury vapor was removed from the reaction system. There was no significant difference in the values obtained in the two cases. With increasing hydrogen chloride concentration the values of  $(k_1/k_2^{1/2})_1$  become progressively greater. The material balance for the conversion of methyl radicals into methane and ethane, as expressed by the ratio  $(C_2H_6 + \frac{1}{2}CH_4)/CO$ , remains similar to that of acetone alone. With hydrogen chloride concentrations considerably larger than those reported in Table I ethane was not detected by the analytical procedure used and the amount of methane formed approached a value about twice that of carbon monoxide without exceeding it. The three determinations with  $\lambda > 2800 \text{ \AA}$  show a similar trend.

Table II shows the analogous experiments at room temperature. It is

TABLE II  
PHOTOLYSIS OF ACETONE-HCl MIXTURES AT ROOM TEMPERATURE  
Effect of HCl concentration

Run	Temp., ° C.	[HCl] <sub>i</sub> , molec./cc. × 10 <sup>-16</sup>	[Ac], molec./cc. × 10 <sup>-18</sup>	Time, sec.	In molec./cc. sec. × 10 <sup>-13</sup>			(k <sub>1</sub> /k <sub>2</sub> <sup>1/2</sup> ) <sub>1</sub> × 10 <sup>9</sup>
					R <sub>CO</sub>	R <sub>CH<sub>4</sub></sub>	R <sub>C<sub>2</sub>H<sub>6</sub></sub>	
Filter 9-54								
49	23.1	....	3.28	4800	0.381	0.024	0.907	...
51	26.4	0.15	3.34	3000	0.343	0.152	0.778	0.3
48	25.4	0.40	3.20	4800	0.215	0.603	0.357	0.8
47	28.8	0.71	3.30	4800	0.238	1.11	0.107	1.5
46	26.7	3.25	3.30	4800	0.238	1.62	0.012	1.5
Filter 0-53								
62	30.1	....	3.23	28800	0.015	0.003	0.018	...
64	28.5	0.53	3.33	50400	0.011	0.039	0.001	0.6
63	30.8	4.31	3.18	64800	0.011	0.043	.....	...

evident that even at room temperature the effect of small hydrogen chloride concentrations is very pronounced. The general trend in the values of  $(k_1/k_2^{1/2})_1$  is similar to that at  $150^\circ$ .

The rates of formation of ethane and methane vary with light intensity, as shown in Table III. It was found that in the photolysis of acetone - hydrogen chloride mixtures at  $150^\circ$  the quantum yield of carbon monoxide formation was close to unity, as in the case of acetone alone. The data on the light absorption in Table III are calculated on the assumption that the quantum yield of carbon monoxide formation is unity. The trend in the ratio of  $R_{CH_4}/R_{C_2H_6}$  is qualitatively the same as in the case of acetone alone and the values of  $(k_1/k_2^{1/2})_1$  become greater as the amount of light absorbed is reduced. The light intensity in these experiments varied as a result of (a) progressive decline in the output of the light source and (b) regulation of the current through the mercury arc. The effects in the two cases were approximately the same.

TABLE III  
PHOTOLYSIS OF ACETONE-HCl MIXTURES (150° C., FILTER 9-54)  
Effect of light intensity

Run	Temp., ° C.	[HCl] <sub>i</sub> , molec./cc. × 10 <sup>-16</sup>	[Ac], molec./cc. × 10 <sup>-18</sup>	Light absorbed, quanta/sec. × 10 <sup>-15</sup>	In molec./cc. sec. × 10 <sup>-13</sup>			C <sub>2</sub> H <sub>6</sub> + $\frac{1}{2}$ CH <sub>4</sub> CO	(k <sub>1</sub> /k <sub>2</sub> <sup>1/2</sup> ) <sub>1</sub> × 10 <sup>9</sup>
					R <sub>CO</sub>	R <sub>CH<sub>4</sub></sub>	R <sub>C<sub>2</sub>H<sub>6</sub></sub>		
32	151.4	0.40	2.66	8.60	7.17	4.61	3.78	0.85	1.1
42	151.2	0.34	2.29	5.60	4.67	3.44	2.11	0.82	1.5
36	152.3	0.36	2.23	4.33	3.61	3.37	1.16	0.80	2.1
70	151.5	0.36	2.39	2.87	2.39	2.67	0.417	0.73	3.0
38	153.3	0.34	2.17	2.03	1.69	2.11	0.262	0.78	3.1

The time of photolysis in all runs was 2400 sec. The amount of light absorbed was calculated on the assumption that the quantum yield of carbon monoxide formation was unity.

The effect of varying time of photolysis is shown in Table IV. There is a distinct decrease in the ratio  $R_{CH_4}/R_{C_2H_6}$  with increased time of photolysis and the values of  $(k_1/k_2^{1/2})_1$  become smaller.

TABLE IV  
PHOTOLYSIS OF ACETONE-HCl MIXTURES (FILTER 9-54)  
Effect of the time of irradiation

Run	Temp., ° C.	Time, sec.	[HCl] <sub>i</sub> , molec./cc. × 10 <sup>-16</sup>	[Ac], molec./cc. × 10 <sup>-18</sup>	In molec./cc. sec. × 10 <sup>-13</sup>			C <sub>2</sub> H <sub>6</sub> + $\frac{1}{2}$ CH <sub>4</sub> CO	(k <sub>1</sub> /k <sub>2</sub> <sup>1/2</sup> ) <sub>1</sub> × 10 <sup>9</sup>
					R <sub>CO</sub>	R <sub>CH<sub>4</sub></sub>	R <sub>C<sub>2</sub>H<sub>6</sub></sub>		
51	26.4	3000	0.15	3.34	0.343	0.152	0.778	2.5	0.29
50	26.2	5700	0.15	3.16	0.350	0.100	0.799	2.4	0.16
52	28.5	9000	0.14	3.16	0.386	0.076	0.835	1.9	0.11
42	151.2	2400	0.34	2.29	4.67	3.44	2.11	0.82	1.5
41	149.2	5400	0.40	2.37	4.59	2.54	2.59	0.81	0.64

#### DISCUSSION

The experimental results on the photolysis of acetone-hydrogen chloride mixtures can be summarized in the following manner: (a) there is a very pronounced effect on addition of relatively small quantities of hydrogen chloride both at 150° and at room temperature: ethane formation is strongly suppressed and the formation of methane is correspondingly increased; (b) the effect is approximately the same at  $\lambda > 2200$  Å and  $\lambda > 2800$  Å; (c) the yield of carbon monoxide and the material balance for the conversion of methyl radicals into methane and ethane are approximately the same as in the case of acetone alone; (d) the products of the photolysis contain no detectable quantities of hydrogen. There is also a decrease in the values of  $(k_1/k_2^{1/2})_1$  with (e) time of photolysis, (f) decreasing hydrogen chloride concentration, and (g) increasing light absorption. At the same time, (h) no significant difference in  $(k_1/k_2^{1/2})_1$  was observed when acetone pressure was reduced from 100 to 50 mm. (This experiment was performed at 150° and  $\lambda > 2200$  Å.)

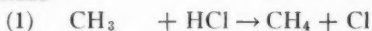
It can be concluded from (e) that hydrogen chloride is at least partially

consumed in the reaction. At the same time, it is evident from the material balance for methane formation that much less than one molecule of hydrogen chloride is used up whenever an additional molecule of methane is formed. This implies either that for the most part hydrogen chloride participates in the reaction only as a second body and is not itself chemically changed, or, if it does undergo a change, that it must be rapidly regenerated. It is difficult to conceive of a plausible mechanism for the first alternative compatible with all the experimental observations.

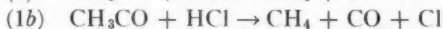
The energetically possible quenching reaction



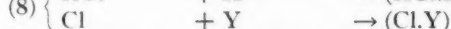
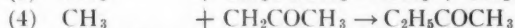
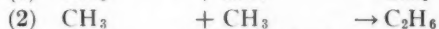
would have to be followed by  $\text{CH}_3\text{CO} \rightarrow \text{CH}_3 + \text{CO}$ . Evidently, it cannot explain complete suppression of ethane formation unless it is assumed that either of the reactions



or



also takes place and is very fast. Furthermore, no kinetic evidence can be derived from the experimental results that reaction (1a) plays an important role. It may, therefore, in the present treatment be disregarded. Since at  $150^\circ$   $\text{CH}_3\text{CO}$  decomposes rapidly into  $\text{CH}_3$  and  $\text{CO}$ , reaction (1) must be of importance. The following scheme of reactions would then seem to be sufficient for a satisfactory explanation of the experimental results:



where (8) is some process by which part of the hydrogen chloride is consumed. Reaction (6) is probably not important below  $200^\circ$ .

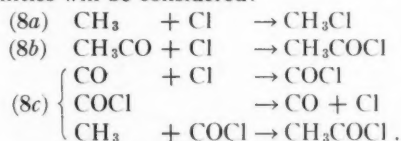
A proof that hydrogen is abstracted from hydrogen chloride to form methane might be furnished by photolysis of deuterated acetone in the presence of relatively large quantities of hydrogen chloride when the process is carried to a small conversion. We thus find that in the photolysis of 100 mm. of deuterated acetone (87%  $d_6$ ) and 30 mm. of hydrogen chloride the methane produced consists predominantly of  $\text{CD}_3\text{H}$ , both at  $150^\circ$  and at room temperature. An eventual spontaneous exchange of H and D between acetone, and hydrogen chloride, even in the case of the most unfavorable redistribution, cannot explain the predominant formation of  $\text{CD}_3\text{H}$ .

The possibility of an appreciable direct photolysis of hydrogen chloride

under conditions of the present experiment seems to be excluded. The long wave length limit of the hydrogen chloride absorption continuum is about 2200 Å (7; 10; 16) and with the filters and the small hydrogen chloride concentrations used no appreciable absorption by hydrogen chloride would be expected. This is substantiated by the lack of hydrogen in the reaction products and the similarity of the observed effects with alternate use of filters 9-54 and 0-53.

The mechanism proposed for the photolysis assumes a partial consumption of hydrogen chloride. A number of chemical processes could explain the decrease in the effective hydrogen chloride concentration, although purely physical phenomena may be also involved to some extent. An assumption that for higher hydrogen chloride concentrations  $[HCl] = \text{Const.} = [HCl]_i$  should, however, be a good approximation, regardless of the nature of these processes.

An approximate treatment of the mechanism of hydrogen chloride consumption can be attempted by assuming that hydrogen chloride concentration is diminished during the process predominantly through a partial removal of free chlorine atoms. A direct abstraction of a chlorine atom from hydrogen chloride is highly unlikely. Results (f) and (g), stated in the summary at the beginning of this section, represent a decrease in the values of  $(k_1/k_2^{1/2})_1$  with an increase in the methyl radical concentration. For simplicity, therefore, only the following possibilities will be considered:



Reactions (8c) are suggested as a possibility on the basis of the investigations of photolysis and thermal decomposition of phosgene (3). If (8c) plays an important part, additions of relatively small quantities of carbon monoxide should inhibit the reaction very strongly. The analytical accuracy in such experiments is somewhat decreased through the presence of additional quantities of carbon monoxide. Nevertheless, it is evident from Table V that (8c) is

TABLE V  
PHOTOLYSIS OF ACETONE-HCl MIXTURES (FILTER 9-54)  
Effect of carbon monoxide concentration

Run	Temp., ° C.	Time, sec.	[CO] <sub>i</sub> , molec./cc. × 10 <sup>-18</sup>	[HCl] <sub>i</sub> , molec./cc. × 10 <sup>-16</sup>	[Ac], molec./cc. × 10 <sup>-18</sup>	In molec./cc. sec. × 10 <sup>-13</sup>			(k <sub>1</sub> /k <sub>2</sub> <sup>1/2</sup> ) <sub>1</sub> × 10 <sup>9</sup>
						R <sub>CO</sub>	R <sub>CH<sub>3</sub></sub>	R <sub>C<sub>2</sub>H<sub>5</sub></sub>	
71	150.1	2400	0.206	...	2.20	(2.36)	0.878	1.28	...
73	150.8	2400	0.160	0.34	2.24	(2.29)	2.21	0.408	2.5
70	151.5	2400	...	0.36	2.27	2.39	2.67	0.417	3.0
74	28.3	7200	0.384	0.44	3.20		0.309	0.190	0.55
75	27.7	7200	...	0.43	3.27	0.136	0.386	0.139	0.73
76	27.5	7200	0.307	3.33	3.24		0.854	0.004	1.3



of little or no importance both at 150° and 25° C. The amounts of carbon monoxide added are in excess of the quantities normally produced in the photolysis under conditions where  $(k_1/k_2^{1/2})_1$  shows a marked decrease.

An exact mathematical treatment of the proposed mechanism, taking into account reactions (8a) and (8b), is at present not possible. Chlorine atom and hydrogen chloride concentrations are mutually related in a very complex way. An assumption of a steady state concentration of  $\text{CH}_3$  and  $\text{Cl}$  implies that there is a mean hydrogen chloride concentration which is smaller than  $\text{HCl}_i$  and remains constant during the photolysis. It can be shown that the value of  $k_1/k_2^{1/2}$  to this new approximation (denoted by  $(k_1/k_2^{1/2})_2$ ) is

$$[2] \quad \left(\frac{k_1}{k_2^{1/2}}\right)_2 = \frac{\frac{R_{\text{CH}_3}}{R_{\text{C}_2\text{H}_5}^{1/2}} - \frac{k_3}{k_2^{1/2}} [\text{Ac}]}{[\text{HCl}]_i - \delta}$$

where

$$\delta = \frac{\left(R_{\text{CH}_3} - \frac{k_3}{k_2^{1/2}} R_{\text{C}_2\text{H}_5}^{1/2} [\text{Ac}]\right)t}{1 + \frac{1}{k' \frac{R_{\text{C}_2\text{H}_5}^{1/2}}{[\text{Ac}]} + k'' \frac{R_{\text{CO}}}{[\text{Ac}]}}$$

and

$$k' = \frac{k_{8a}}{k_7 k_2^{1/2}}, \quad k'' = \frac{k_{8b}}{k_7 k_{11}}, \quad \text{Ac} = \text{CH}_3\text{COCH}_3,$$

$t$  is the time of photolysis.

Equation [2] is in qualitative agreement with the experimental observations. At 150°  $k_{11}$  is very large and as a further simplification  $k''$  can be neglected. A simultaneous plot of quantum yields of methane and ethane formation at 150° and  $\lambda > 2200 \text{ \AA}$  for varying initial hydrogen chloride concentrations, other experimental factors being approximately constant, is shown in Fig. 1. Acetone pressure in all cases was close to 100 mm. By plotting also the remaining experimental data at 150° and  $\lambda > 2200 \text{ \AA}$  it is found that all the points either fall on the pair of curves (quantum yields of methane and ethane respectively) or can be brought thereon through a simple shift in hydrogen chloride concentration. Thus there is an indication that  $k_1/k_2^{1/2} = f([\text{HCl}]_i - \Delta)$ . Equation [2] is a very simplified expression of this functional dependence and an accurate agreement with the experimental results cannot be expected. By taking  $k' = 4.2 \times 10^{10}$  and  $k'' = 0$  the drifts observed at 150° and  $\lambda > 2200 \text{ \AA}$  largely disappear and the mean value of  $(k_1/k_2^{1/2})_2$  is about  $4.5 \times 10^{-9}$ . However, there remains a large scatter of the values. Also, this treatment fails with the results obtained at room temperature, though the values of  $k_1/k_2^{1/2}$  can be mutually approached.

Drifts in the values of  $(k_1/k_2^{1/2})_1$  make an accurate evaluation of the activation energy of reaction (1) impossible. On the basis of the proposed mechanism extrapolations to  $t = 0$ ,  $I_a = 0$ , or to large hydrogen chloride concentrations should supply the true values of  $k_1/k_2^{1/2}$ . Limitations in the accuracy of the analytical procedure make these extrapolations unreliable. It seems possible,

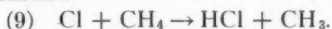
however, to obtain an approximate estimate of the activation energy of the reaction from the data in Tables I and II. With increasing hydrogen chloride concentrations  $(k_1/k_2^{1/2})_1$  tends to a value of about  $4.5 \times 10^{-9}$  at  $150^\circ$  and  $1.5 \times 10^{-9}$  at  $28^\circ$  C. From the ratio of the two values, and taking into account the  $T^{1/2}$  factor in the pre-exponential term, 2.1 kcal. per mole is obtained for  $E_1 - \frac{1}{2} E_2$ . Assuming mean collision diameters of 3.5 Å and 1.3 Å for  $\text{CH}_3$  and hydrogen chloride respectively,  $P_1/P_2^{1/2}$  is found to be  $6.7 \times 10^{-3}$ . With unity as a plausible value for  $P_2$  (6), the value of  $P_1$  becomes  $6.7 \times 10^{-3}$ . In view of the uncertainty of the extrapolation to high hydrogen chloride concentrations, an uncertainty of  $\pm 1$  kcal. per mole in the above value of  $E_1 - \frac{1}{2} E_2$  would seem to be a sufficiently conservative estimate. This introduces a factor of 3.3 as the corresponding uncertainty in the value of  $P_1/P_2^{1/2}$ .

The present estimate of the activation energy of the reaction (1) is based on the values of rate constants at  $150^\circ$  and  $28^\circ$  C. In the photolysis of acetone alone two distinct values of the activation energy of hydrogen abstraction by methyl radicals have been reported: 9.7 kcal. per mole between approximately  $120^\circ$  and  $300^\circ$  (15) and 6.1 kcal. per mole between  $120^\circ$  and  $25^\circ$  (5). There is still no complete explanation of this apparent anomaly. This discrepancy, however, should not affect the estimated value of the activation energy of reaction (1) because in the extrapolation of  $(k_1/k_2^{1/2})_1$  to large hydrogen chloride concentrations the contribution of the reaction (3) becomes negligibly small. At the same time, Equation [1] should be valid regardless of the discrepancy in the value of  $E_3 - \frac{1}{2} E_2$  provided methane and ethane are formed by a free radical mechanism. Dorfman and Noyes (5) have shown that the experimental data on photolysis of acetone are fully compatible with the free radical mechanism both at  $25^\circ$  and at higher temperatures. Benson and Falterman (1) have recently investigated the photolysis of equimolecular mixtures of ordinary and deuterated acetone and found that both at  $35^\circ$  and at higher temperatures  $\text{C}_2\text{D}_6$ ,  $\text{CD}_3\text{CH}_3$ , and  $\text{C}_2\text{H}_6$  are formed in the ratio of 1:2:1, as would be expected in the case of a free radical mechanism. In analogous experiments we find that both at  $25^\circ$  and  $150^\circ$  several times as much  $\text{CD}_3\text{H}$  is formed as  $\text{CD}_4$ . It would seem, therefore, that at room temperature as well as at higher temperatures methane and ethane are formed by a free radical mechanism. Nevertheless, it is possible that an eventual explanation of the curvature of the Arrhenius plot for acetone photolysis at lower temperatures may necessitate a revision of the conclusions suggested here.

The value of the steric factor obtained for reaction (1) seems to be in agreement with the values previously observed for similar types of reactions. It is also of interest that from the experimental results of Williams and Ogg (17) a value of 2.4 kcal. per mole is obtained for  $E_1$  if it is assumed that the reaction of methyl radicals with molecular iodine requires no activation energy. These authors, however, suggest that under the conditions of their experiments (large hydrogen chloride concentrations) a reaction of "hot" methyl radicals with hydrogen chloride is of importance. On the other hand, in the experiments reported in the present paper, the hydrogen chloride concentrations used were very small and, consequently, any "hot" methyl

radicals were thermalized by collisions with acetone before reacting with hydrogen chloride, so that a reaction of "hot" methyl radicals is unlikely to be important.

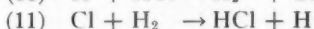
The low value of the activation energy obtained for reaction (1) may appear at first sight somewhat surprising in view of the value of more than 9 kcal. for the reaction of methyl radicals with hydrogen (11). A consideration of bond strengths shows that reaction (1) is nearly thermoneutral or only slightly endothermic.  $E_1$  would then be expected to be comparable with the activation energy of the reverse reaction



For the comparable set of reactions (which are also approximately thermoneutral)



and



Bodenstein (2) estimates collision yields of the same order of magnitude,  $10^{-4}$ . Tamura (14), using the method of thermal analysis of reaction velocity (8), has investigated reaction (9) at 19° C. and obtained a collision yield of  $2.4 \times 10^{-5}$  and, consequently,  $E_9 \leq 6.2$  kcal. per mole. The maximum value of 6.2 kcal. per mole corresponds to a steric factor of unity; for a steric factor of  $10^{-3}$   $E_9$  reduces to 2.1 kcal. per mole. Taking  $E_2 = 0$ ,  $P_2 = 1$ , and mean molecular diameters of 3.5 Å and 1.3 Å for  $\text{CH}_3$  and hydrogen chloride respectively, we obtain for reaction (1) at 28° a collision yield of  $2.0 \times 10^{-4}$ , which leads to a value of  $E_1 \leq 5.1$  kcal. per mole. There is, therefore, an approximate agreement between the collision yields of reactions (1) and (9). The consistency of the value obtained for  $E_1$  and Tamura's value for  $E_9$  is thus seen to be uncertain to the extent of the uncertainty of the steric factor of the latter reaction. Unfortunately, most reactions involving chlorine atoms have been studied at a single temperature. Rodebush and Klingelhoefer (13) have investigated reaction (11) at 25° and 0° C. Chlorine atoms were produced by electrodeless discharge and their concentration measured by a gauge similar to the Wrede gauge. From the experimental measurements reported and the uncertainty suggested by the authors, it would seem that  $P_{11}$  is between  $10^{-2}$  and unity. However, in view of the relatively small temperature range employed, we do not feel justified in drawing any definite conclusions relevant to the present work.

Reaction between methyl radicals and hydrogen chloride is seen to be fast enough so that the presence of even relatively small quantities of hydrogen chloride may have a pronounced effect on the over-all course of a reaction involving methyl radicals. Actually,  $k_2$  is probably about ten thousand times as great as  $k_1$  at room temperature and the pronounced catalytic effect of hydrogen chloride is only due to the extremely small  $\text{CH}_3$  concentrations and to the rapid regeneration of hydrogen chloride. The action of hydrogen chloride may manifest itself in most cases solely in an increased production of methane and almost complete suppression of ethane without affecting in any other way the over-all composition of the products. Under such conditions the  $\text{CH}_3$  concentration is so much suppressed that only negligible

quantities of  $\text{CH}_3\text{Cl}$  result from reaction (8a) and may not be analytically detectable. Thus, in the photolysis of mixtures of acetone and chlorinated hydrocarbons, hydrogen chloride may be a product of the reaction or could be present as an impurity. The strong suppression of ethane formation could be in such cases predominantly due to a very fast abstraction of hydrogen from the hydrocarbon. At the same time, to a smaller or greater extent, the effect may be due to a fast abstraction of hydrogen via hydrogen chloride. The relative importance of the two possibilities can be ascertained from quantitative considerations when hydrogen chloride concentration is known, or by a suitable selective inhibition of the hydrogen chloride effect (4). The significance of the catalytic effect of hydrogen chloride in the apparent carbon tetrachloride anomaly mentioned in the introduction, and in some other instances, will be discussed elsewhere in this Journal (4) on the basis of additional experiments performed.

#### ACKNOWLEDGMENT

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## PHOTOLYSIS OF MIXTURES OF ACETONE AND SOME CHLORINATED METHANES<sup>1</sup>

BY R. J. CVETANOVIĆ,<sup>2</sup> F. A. RAAL,<sup>3</sup> AND E. W. R. STEACIE

### ABSTRACT

Additions of carbon tetrachloride to acetone show in photolysis an apparent anomaly: methane formation is strongly increased and the formation of ethane correspondingly lowered. In this manner a hydrogen abstraction reaction is simulated. The cause of the anomaly and its bearing on the photolysis of acetone with other chlorinated methanes has been investigated.

### INTRODUCTION

The reaction of methyl radicals, formed by the photolysis of acetone, with several partially halogenated methanes has recently been investigated by two of the authors (7). Rates of production of methane, ethane, and carbon monoxide were determined between 110° and 220°C. By analogy with the well investigated photochemical reactions in acetone-hydrocarbon mixtures (11) it was assumed that all ethane was produced by combination of methyl radicals and all methane by hydrogen abstraction from acetone and from the added halogenated methane. With this assumption, and assuming also time independent concentrations of methyl radicals, acetone, and the halogenated methane, it was possible to evaluate the activation energies of the investigated reactions relative to the activation energy of the combination of methyl radicals, as well as their steric factors. In this manner it was shown that the ease of abstraction of a hydrogen atom from a halogenated methane increased as the number of halogen atoms in the compound increased.

In all the investigated reactions the addition of the partially halogenated methane to acetone had the effect of increasing the rate of formation of methane with a corresponding suppression of ethane formation. It seemed reasonable to suppose that the increased methane formation was entirely due to the additional abstraction of hydrogen from the halogenated methane. Thus in the series  $\text{CH}_3\text{Cl}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{CHCl}_3$ , the effect became more pronounced as the number of the chlorine atoms in the molecule increased, indicating that the rates of hydrogen abstraction became faster. The last member of the series, carbon tetrachloride, contains no hydrogen atoms and its addition to acetone should not have any effect on the rates of methane production. It was thought, therefore, that the photolysis of a mixture of acetone and carbon tetrachloride would provide a suitable test for the correctness of the assumptions made. Early experiments, however, showed that additions of carbon tetrachloride to acetone produced an effect entirely analogous to that produced by chloroform and possibly even more pronounced. There was, therefore, a continuous increase in the intensity of the

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<sup>2</sup> National Research Council of Canada Postdoctorate Fellow. Present address: Division of Applied Chemistry, National Research Council, Ottawa, Canada.

<sup>3</sup> National Research Council of Canada Postdoctorate Fellow. Present address: National Chemical Research Laboratory, Pretoria, South Africa.



effect along the series without the expected disappearance in the case of carbon tetrachloride. Since a direct hydrogen abstraction cannot be postulated in the case of carbon tetrachloride, the question of immediate interest is whether the observed effect is of identical nature for all the members of the series, in which case hydrogen abstraction would not suffice to explain it; or otherwise, is the carbon tetrachloride anomaly peculiar to this compound alone without affecting the other members of the series.

Although in all the experiments performed the best available grades of the reagents were used, it was necessary to investigate first the possibility that the anomalous behavior of carbon tetrachloride may be due to the presence of some impurity. Since the effect produced by carbon tetrachloride is at least as pronounced as that of any other member of the series, it is evident that the presence of even large quantities of the other chlorinated methanes cannot explain the observed anomaly. If an impurity is responsible for the large increase in methane production, small additions of it directly to acetone should produce an analogous effect. In a largely qualitative experiment we have observed that additions of relatively small amounts of hydrogen chloride to acetone do suppress strongly ethane formation with a corresponding large increase in the amount of methane formed. In order to be able to draw any unambiguous conclusions regarding the importance of a possible hydrogen chloride impurity in carbon tetrachloride, it was necessary to know how small the quantities of hydrogen chloride are which are still sufficient to produce an appreciable effect. This information is now available (2), and it will be evident from the subsequent discussion, based on a number of experiments performed in order to clarify this point, that a hydrogen chloride impurity alone cannot explain the carbon tetrachloride effect. However, the possibility that certain amounts of hydrogen chloride may be formed during the photolysis itself has been further investigated and the results arrived at seem to offer a satisfactory explanation of the apparent anomaly. In the present paper we wish to report the results of the experiments performed and their bearing on the photolysis of mixtures of acetone and the other members of the series of chlorinated methanes.

## EXPERIMENTAL

### *Apparatus*

The apparatus, the analysis system, and the optical system used have been described before (2), as well as the procedure employed for mixing the reactants. Additional facility was provided for freezing over into sampling traps the condensable fraction of the products without an appreciable contamination with mercury, the latter having been found to obscure to some extent qualitative tests for hydrogen chloride. In order to decrease the solubility of some of the reactants in stopcock grease, a commercial silicone grease was used in the relevant parts of the reaction system.

The light from a Hanovia S-500 medium pressure arc was roughly collimated by a highly polished aluminum cylinder. Two filters were alternately included in the optical system: a Corning 9-54 filter with a cutoff below about 2200 Å and a Corning 0-53 filter with a cutoff below about 2800 Å. The cylindrical silica re-



action cell had a volume of 120 ml. and during a photolysis was completely filled with the light beam.

The aluminum block furnace used was heated by the current supplied from a Sorensen voltage regulator and its temperature was maintained to within about 1°C.

### Materials

Acetone was a Mallinckrodt analytical reagent product, refluxed over potassium permanganate, and bulb to bulb distilled *in vacuo*. The same grade acetone, dried over anhydrous sodium sulphate and subjected to repeated bulb to bulb distillation *in vacuo*, without previous permanganate treatment, has also been used without any appreciable difference in the results.

Anachemia and Mallinckrodt reagent grades carbon tetrachloride were alternately used. The methods employed for further purification of carbon tetrachloride will be referred to in the following section.

Chloroform was a reagent grade Anachemia product. It was used alternately (a) without drying before the vacuum distillation, (b) dried over anhydrous sodium sulphate, and (c) dried over phosphorus pentoxide. In all cases it was finally subjected to repeated bulb to bulb distillation *in vacuo*.

Ammonia was a Matheson Company product. Normal pentane was a pure grade Phillips product, 99 mole % minimum. These compounds were further purified before their use by repeated bulb to bulb distillation *in vacuo*.

### RESULTS

In early experiments with acetone and carbon tetrachloride the reagent grade carbon tetrachloride was only subjected to repeated bulb to bulb distillation *in vacuo* prior to its use. In order to test whether a hydrogen chloride impurity was responsible for the observed increased methane production, a sample of the carbon tetrachloride was thoroughly washed 10 times with distilled water, dried over a reagent grade anhydrous sodium sulphate, distilled through a column, and finally bulb to bulb distilled *in vacuo*. This treatment failed to affect the observed rates of methane and ethane formation, although on the basis of the results from photolysis of acetone-hydrogen chloride mixtures (2) the calculated quantities of hydrogen chloride required to produce an analogous effect would have had in some cases to constitute an impurity of several per cent. Evidently, if hydrogen chloride was the cause of the apparent anomaly, it could not have been introduced in such quantities as an impurity but must have been formed subsequently. This is further substantiated by the investigation of the effect of additions of small quantities of ammonia to acetone-carbon tetrachloride mixtures before their photolysis at 150°C. When 1 mm. of hydrogen chloride is added to 100 mm. of acetone and the mixture is photolyzed at 150°, the amount of ethane produced is negligibly small and the amount of methane formed becomes nearly twice the amount of carbon monoxide. On the other hand, if to such a mixture somewhat more than 1 mm. of ammonia is added and all the reactants are condensed together outside the reaction cell and then expanded, the rates of formation of methane and ethane become, in the subsequent photolysis, the same as in the case of pure acetone alone. The pretreatment by ammonia

removes hydrogen chloride from the gaseous mixture. A similar pretreatment by ammonia of mixtures of acetone and carbon tetrachloride, however, fails to remove the effect due to carbon tetrachloride as is evident from Table I. The

TABLE I  
PHOTOLYSIS OF ACETONE- $\text{CCl}_4$  MIXTURES AT  $150^\circ\text{C}$ .  
EFFECT OF ADDITION OF AMMONIA

Run	Temp., °C.	Time, sec.	In molec./cc. $\times 10^{-18}$			In molec./cc. sec. $\times 10^{-13}$			$R_{\text{CH}_4}$
			[Ac]	[CCl <sub>4</sub> ]	[NH <sub>3</sub> ]	$R_{\text{CO}}$	$R_{\text{CH}_4}$	$R_{\text{C}_2\text{H}_6}$	$\left(\frac{R_{\text{C}_2\text{H}_6}}{R_{\text{CH}_4}}\right)^{\frac{1}{2}}$ $\times 10^{-6}$
Filter 0-53									
7	153	7200	2.29	—	—	0.408	0.346	0.171	2.6
5	153	7200	2.29	0.18	0.02	0.486	0.598	0.062	7.6
4	153	7200	2.29	0.27	0.03	0.439	0.614	0.078	7.0
6	153	7200	2.29	0.23	0.09	0.424	0.583	0.047	8.5
136	151	25200	2.27	0.02 <sub>2</sub>	—	0.211	0.331	0.018	7.0
Filter 9-54									
8	152	3600	2.30	—	—	4.90	1.57	3.74	2.6
11	152	3600	2.30	—	0.05	4.34	1.51	3.30	2.6
10	154	3600	2.28	0.24	—	3.84	5.78	0.405	28.7
9	153	3600	2.31	0.20	0.18	5.03	5.81	0.816	20.3

amounts of ammonia added were in all cases in large excess of the calculated quantity of hydrogen chloride corresponding to the observed effect. At the same time, comparable quantities of ammonia had no appreciable effect on the photolysis of acetone alone, as is seen from Expt. 11; this is in agreement with the previous investigation of the photolysis of acetone-ammonia mixtures by Trotman-Dickenson and Steacie (12) who found an activation energy of 10.0 kcal. per mole and a steric factor of  $6 \times 10^{-4}$  for the abstraction of hydrogen from ammonia by methyl radicals. In the blank experiments conducted with acetone-carbon tetrachloride mixtures at  $150^\circ$  and differing only in the omission of irradiation, no formation of carbon monoxide, methane, or ethane could be detected; the lack of the usual products shows that under such conditions there are no thermal effects of any importance.

It is evident that the supposition that a hydrogen chloride impurity is solely responsible for the observed anomalous behavior of carbon tetrachloride can be discounted. There remains, however, the possibility that small amounts of hydrogen chloride are formed in the photolysis. The amounts of hydrogen chloride formed would normally be expected to be rather small and their detection in the presence of very large quantities of the reactants and the other products may offer some difficulty. A series of qualitative experiments at  $150^\circ$  has, however, shown that hydrogen chloride is formed when mixtures of acetone and carbon tetrachloride are irradiated, both at  $\lambda > 2200 \text{ \AA}$  and  $\lambda > 2800 \text{ \AA}$ . Also at room temperature ( $\lambda > 2200 \text{ \AA}$ ) hydrogen chloride could be detected. In these experiments large quantities of carbon tetrachloride (up to 40 mm.) and 70 to 80 mm.

of acetone were used and the irradiations extended for periods of 6 to 12 hr. The condensable products and the excess of the reactants were frozen over into a sampling trap containing a small amount of silver nitrate solution. On warming, distinct white precipitates, soluble in ammonia and reprecipitated by nitric acid, were formed. Blank experiments with identical treatment except that the irradiation was omitted showed no silver chloride precipitates. Also, the bulk liquid of the carbon tetrachloride used gave a negative test.

Since hydrogen chloride is a product in the photolysis of acetone-carbon tetrachloride mixtures, it must, in view of its pronounced catalytic action (2), be responsible for at least a part of the observed apparent anomaly. The experiments with addition of ammonia show that at 150° there is at the best only a slight inhibition of the carbon tetrachloride effect. This might be due to a slow removal of hydrogen chloride formed in the reaction because at 150° ammonium chloride would be dissociated to an appreciable extent. On the other hand, small amounts of ammonia added to acetone-carbon tetrachloride mixtures photolyzed at room temperature completely inhibit the anomalous effect and the rates of formation of methane and ethane become the same as in the photolysis of acetone alone.

The effect of additions of carbon tetrachloride to acetone photolyzed at  $\lambda > 2200 \text{ \AA}$  is summarized in Tables II and III. Variations with carbon tetra-

TABLE II  
PHOTOLYSIS OF ACETONE- $\text{CCl}_4$  MIXTURES (FILTER 9-54)  
EFFECT OF  $\text{CCl}_4$  CONCENTRATION

Run	Temp., °C.	Time, sec.	In molec./cc. $\times 10^{-13}$		In molec./cc. sec. $\times 10^{-13}$			$R_{\text{CH}_4}$	$\text{C}_2\text{H}_6 + \frac{1}{2}\text{CH}_4$	$\gamma_{\text{CCl}_4} \times 10^{11}$
			[Ac]	[CCl <sub>4</sub> ]	R <sub>CO</sub>	$R_{\text{CH}_4}$	$R_{\text{C}_2\text{H}_6}$	$\frac{(\text{R}_{\text{C}_2\text{H}_6})^{1/2} \times 10^{-4}}{\text{CO}}$	CO	
150°C.										
8	152	3600	2.30	—	4.90	1.57	3.74	2.6	0.92	—
13	149	3600	2.29	0.023	3.88	3.28	1.55	8.4	0.82	25.2
89	150	3000	2.41	0.030	5.61	4.52	1.70	11.0	0.80	28.3
88	147	3000	2.32	0.078	5.60	6.10	0.979	19.5	0.72	22.0
87	150	3000	2.41	0.086	6.26	7.44	0.860	25.4	0.73	26.6
12	152	3600	2.32	0.18	3.99	5.17	0.536	22.3	0.78	13.7
10	154	3600	2.28	0.24	3.84	5.78	0.405	28.7	0.86	10.9
24	151	3600	2.41	0.39	5.43	8.22	0.252	51.8	0.80	12.6
86	151	2400	2.44	0.55	7.73	11.2	0.551	47.8	0.80	8.2
14	152	3600	2.37	0.75	3.22	5.84	0.093	60.5	0.94	7.7
Room temperature										
91	26	3600	3.33	—	0.496	0.041	1.15	0.12	2.4	—
90	30	3600	3.32	0.12	0.474	0.316	0.910	1.0	2.3	0.8
96	30	3390	3.24	0.45	0.368	0.946	0.371	4.9	2.3	1.1
95	28	3600	3.06	1.24	0.295	1.32	0.161	10.4	2.8	0.8

TABLE III  
PHOTOLYSIS OF ACETONE- $\text{CCl}_4$  MIXTURES (ROOM TEMP., FILTER 9-54)  
EFFECT OF TIME OF IRRADIATION

Run	Temp., °C.	Time, sec.	In molec./cc. $\times 10^{-13}$		In molec./cc. sec. $\times 10^{-13}$			$R_{\text{CH}_4}$ $(R_{\text{C}_2\text{H}_6})^{1/2} \times 10^{-4}$	$\text{C}_2\text{H}_6 + \frac{1}{2}\text{CH}_4$ CO	$\gamma_{\text{CCl}_4} \times 10^{11}$
			[Ac]	$[\text{CCl}_4]$	R <sub>CO</sub>	$R_{\text{CH}_4}$	$R_{\text{C}_2\text{H}_6}$			
91	26	3600	3.33	—	0.496	0.041	1.15	0.12	2.4	—
93	27	1800	3.24	0.122	0.496	0.365	0.941	1.2	2.3	0.9
90	30	3600	3.32	0.120	0.474	0.316	0.910	1.0	2.3	0.8
92	30	7200	3.27	0.125	0.458	0.414	0.732	1.5	2.1	1.1
94	28	10800	3.18	0.130	0.388	0.366	0.621	1.4	2.1	1.0

chloride concentration at 150° and at room temperature are shown in Table II. There is a pronounced increase in the rate of formation of methane and a decrease in production of ethane with increasing carbon tetrachloride concentrations. On the other hand, variation of time of irradiation in the series of experiments in Table III, conducted at room temperature and at  $\lambda > 2200 \text{ \AA}$ , has little or no effect on rates of methane and ethane formation. At the same time, the rates of carbon monoxide formation remained in all cases approximately the same as in the photolysis of acetone alone under comparable conditions. An eventual slight variation in the carbon monoxide production could, however, remain unnoticed because of a gradual decline in the amount of light absorbed due to decreasing transparency of the reaction cell window.

#### DISCUSSION

There seems to be evidence that a halogenated methane molecule can undergo decomposition into a halogen atom and a radical either as a result of direct absorption of a suitable light quantum (4; 5) or by quenching some excited molecule (1) or atom (6). If it can be assumed that in the present experiments carbon tetrachloride undergoes a direct photolysis or an acetone photosensitized decomposition, the formation of hydrogen chloride can be easily explained by subsequent reaction of chlorine atoms with acetone. These processes need take place to only a relatively small extent in order to account for the quantity of hydrogen chloride necessary to explain the carbon tetrachloride effect.

If small amounts of hydrogen atoms appear in acetone photolysis, a hydrogen chloride formation could be attributed to direct abstraction of chlorine from carbon tetrachloride. In the absence of carbon tetrachloride small amounts of hydrogen would thus be formed. While limitations in the mass-spectrometer analysis prevented an unambiguous detection of traces of hydrogen in the products of acetone photolysis, its quantity, if present, would be too small to account for the amounts of hydrogen chloride necessary to explain the carbon tetrachloride effect under conditions of the present experiments.

In order to obtain some insight into the mode of formation of hydrogen chloride a number of experiments were performed with carbon tetrachloride and *n*-pentane, the latter serving only as a supplier of hydrogen. Mixtures of 30 to 40 mm. carbon tetrachloride and about 80 mm. *n*-pentane were irradiated at 150° for 8 to 12 hr. and the products tested qualitatively for hydrogen chloride. Some hydrogen chloride was found when filter 9-54 was used, as was to be expected in view of the characteristics of the filter and the threshold for the long wave length continuous absorption of carbon tetrachloride (5). With filter 0-53, on the other hand, no hydrogen chloride could be detected by the analytical procedure used, although under analogous conditions with mixtures of carbon tetrachloride and acetone, hydrogen chloride was formed. It appears, therefore, that an acetone photosensitized decomposition of carbon tetrachloride does take place and any scheme that may be proposed to explain the carbon tetrachloride effect in acetone photolysis would have to take it into account. It is likely that in the process chlorine atoms are liberated and then abstract hydrogen from acetone to form hydrogen chloride.

In the photolysis of acetone - hydrogen chloride mixtures (2) methane and ethane are formed by the reactions

- (1)  $\text{CH}_3 + \text{HCl} \rightarrow \text{CH}_4 + \text{Cl}$ ,
- (2)  $\text{CH}_3 + \text{CH}_3 \rightarrow \text{C}_2\text{H}_6$ ,
- (3)  $\text{CH}_3 + \text{CH}_3\text{COCH}_3 \rightarrow \text{CH}_4 + \text{CH}_2\text{COCH}_3$ .

The following reactions are generally believed also to take place in the photolysis of acetone

- (4)  $\text{CH}_3 + \text{CH}_2\text{COCH}_3 \rightarrow \text{C}_2\text{H}_5\text{COCH}_3$ ,
- (5)  $2 \text{CH}_2\text{COCH}_3 \rightarrow (\text{CH}_2\text{COCH}_3)_2$ ,
- (6)  $\text{CH}_2\text{COCH}_3 \rightarrow \text{CH}_2\text{CO} + \text{CH}_3$ ,

the last reaction being of importance at temperatures above 200 to 250°. Chlorine atoms liberated in reaction (1) can react with acetone to regenerate hydrogen chloride.

- (7)  $\text{Cl} + \text{CH}_3\text{COCH}_3 \rightarrow \text{HCl} + \text{CH}_2\text{COCH}_3$ .

At the same time, the HCl regeneration is found to be incomplete, probably largely owing to the reaction

- (8)  $\text{CH}_3 + \text{Cl} \rightarrow \text{CH}_3\text{Cl}$ .

In the photolysis of acetone - carbon tetrachloride mixtures  $\text{CCl}_3$  radicals are probably present and may participate in a variety of reactions (10). It is, therefore, possible that chlorine atoms may also be consumed by combination with  $\text{CCl}_3$  radicals or with some of the products of reactions involving these radicals. This effect would tend to be greater with increasing carbon tetrachloride concentration.

Regardless of the mode of primary formation of hydrogen chloride, its concentration could be expected to reach a steady state, at least under conditions where the primary formation is relatively slow. As a first approximation the hydrogen chloride steady state concentration may be considered proportional to the concentration of carbon tetrachloride. The usual steady state treatment shows then that a quantity

$$[1] \quad \gamma_{\text{CCl}_4} = \frac{\frac{R_{\text{CH}_4}}{(\text{RC}_2\text{H}_6)^{1/2}} - \frac{k_3}{k_2^{1/2}} [\text{Ac}]}{[\text{CCl}_4]}$$

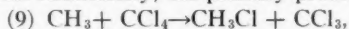
should be constant at a constant temperature. The values of  $\gamma_{\text{CCl}_4}$  are given in the last column of Tables II and III and are seen to vary relatively little with time of irradiation (Table III). There is, however, a decrease in  $\gamma_{\text{CCl}_4}$  by a factor of 3 to 4 with increasing carbon tetrachloride concentrations at 150° and  $\lambda > 2200 \text{ \AA}$  (Table II).

Equation [1] evidently does not take into account some of the important features of the process considered here. With the available information a more elaborate kinetic treatment could hardly be justifiable, especially in view of the unknown nature of the primary carbon tetrachloride decomposition. It should, however, be mentioned that drift in  $\gamma_{\text{CCl}_4}$  values in Table II can be explained by an eventual increasing Cl atom consumption with increasing carbon tetrachloride concentration, as indicated previously.

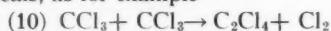
Initial decomposition of carbon tetrachloride may be due to some energy



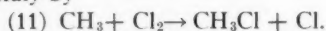
transfer process. Alternately, the primary process may be the reaction



in which case chlorine atoms would have to result from some secondary reaction of  $\text{CCl}_3$  radicals, as for example



followed rapidly by



Schumacher and Wolff (8) failed to detect any  $\text{C}_2\text{Cl}_6$  in the products of the reaction of chlorine atoms with chloroform, although formation of  $\text{CCl}_3$  radicals seems to be well established. Reaction (9) is analogous to the reaction of hydrogen atoms with carbon tetrachloride, for which Vance and Baumann (13) find an activation energy of 3.5 kcal. per mole and a steric factor of 0.007. In view of the large difference in the bond strengths of the products in the two reactions, reaction (9) would probably be considerably slower. Vance and Baumann find that with the lowest carbon tetrachloride concentrations used in their experiments the number of moles of hydrogen chloride formed is in some cases more than twice the number of moles of carbon tetrachloride originally present. They therefore postulate, besides the chlorine abstraction by hydrogen atoms from carbon tetrachloride, an additional abstraction from  $\text{CCl}_3$ . Smyser and Smallwood (9) and the later workers have observed formation of a solid with high chlorine content, possibly a polymer of  $\text{C}_2\text{Cl}_4$ . In the present experiments a slight deposit on the walls of the reaction vessel was also observed, although its nature is not known.

Before additional experimental information is acquired, any attempt to distinguish between different possible mechanisms would be highly speculative, although it is possible on the basis of hypothetical reactions to derive approximate expressions fitting the experimental data reasonably well. It is hoped that as a result of the experiments now in progress with other halogenated methanes, it may become possible to propose, with less ambiguity, an explanation of the mode of hydrogen chloride formation.

Equation [1] is formally identical with the expression generally used for calculating rate constants of hydrogen abstraction reactions. It gives the value of an apparent rate constant (divided by  $k_2^{1/2}$ ) for the hydrogen abstraction reaction, which is, in the case of carbon tetrachloride, meaningless. The approximate constancy of  $\gamma$  values obtained for carbon tetrachloride under similar conditions at a single temperature illustrates the danger that such an apparent value could be mistakenly identified with the rate constant of the abstraction reaction in the case of a compound where the abstraction is possible. A question of interest, is, therefore, to what extent the other chlorinated methanes are affected by the anomaly observed in the case of carbon tetrachloride.

Additional experiments were carried out with acetone-chloroform mixtures at  $150^\circ$  and at room temperature. Hydrogen chloride was identified qualitatively as a product in the photolysis at room temperature and with the filter 9-54. Moreover a sample of chloroform purified only by repeated bulb to bulb distillation gave under similar conditions a positive test for hydrogen chloride even in the absence of acetone. In both cases irradiation was essential for hydrogen



chloride formation. The effect of additions of chloroform to acetone photolyzed at room temperature both at  $\lambda > 2200 \text{ \AA}$  and  $\lambda > 2800 \text{ \AA}$  was quite pronounced, comparable in magnitude with that of carbon tetrachloride. The values of  $\gamma_{\text{CHCl}_3}$ , calculated from the expression analogous to Equation [1], showed certain variations with the experimental conditions although the number of determinations was not sufficient to establish definite correlations. At  $150^\circ$  the values of  $\gamma_{\text{CHCl}_3}$  were found to be considerably larger than previously reported (7), probably partly owing to different light intensities used but mainly owing to an improved mixing of the reactants in the present experiments. The values of  $\gamma_{\text{CHCl}_3}$  obtained for photolysis at  $150^\circ$  are comparable in magnitude with the corresponding values of  $\gamma_{\text{CCl}_4}$ .

It has been mentioned previously that at room temperature the effect due to carbon tetrachloride can be completely inhibited with relatively very small amounts of ammonia. In the case of chloroform, as shown in Table IV, com-

TABLE IV  
PHOTOLYSIS OF ACETONE- $\text{CCl}_4$  AND ACETONE- $\text{CHCl}_3$  MIXTURES (FILTER 9-54)  
EFFECT OF ADDITION OF AMMONIA AT ROOM TEMPERATURE

Run	Temp., °C.	Time, sec.	In molec./cc. $\times 10^{-18}$			In molec./cc. sec. $\times 10^{-12}$			$\frac{R_{\text{CH}_4}}{(R_{\text{C}_2\text{H}_6})^{\frac{1}{2}} \times 10^{-4}}$	$\gamma \times 10^{11}$
			[Ac]	[Cl]	[NH <sub>3</sub> ]	R <sub>CO</sub>	R <sub>CH<sub>4</sub></sub>	R <sub>C<sub>2</sub>H<sub>6</sub></sub>		
Acetone and carbon tetrachloride										
139	28	32400	3.21	0.257	—	0.135	0.415	0.078	4.7	1.8
141	28	27000	3.21	0.226	0.02	0.086	0.010	0.150	0.1	—
140	28	34200	3.20	0.217	0.28	0.118	0.017	0.220	0.1	—
137	26	32400	3.25	0.237	0.33	0.271	0.028	0.271	0.1	—
91	26	3600	3.33	—	—	0.496	0.041	1.15	0.1	—
Acetone and chloroform										
142	30	3600	1.85	1.44	—	0.438	1.61	0.274	9.8	0.67
145	28	3600	1.81	1.36	0.03	0.315	0.679	0.479	3.1	0.22
143	31	3600	1.71	1.36	0.27	0.462	0.935	0.654	3.7	0.26
144	30	3600	1.91	1.34	1.22	0.407	0.804	0.545	3.4	0.25

parable amounts of ammonia suppress only partly the additional formation of methane and further additions of ammonia, up to relatively large concentrations, fail to produce any further inhibition. It may be justifiable to assume that under these conditions the part of the effect due to the presence of hydrogen chloride is completely inhibited. The residual effect can then be ascribed to the reaction



and the approximately constant value of  $\gamma_{\text{CHCl}_3} = 0.24 \times 10^{-11}$  can be identified with  $k_{12}/k_2^{1/2}$ . Assuming as plausible values (3)  $P_2 = 1$  and  $E_2 = 0$  and taking mean collision diameters of  $3.5 \text{ \AA}$  and  $5 \text{ \AA}$  for  $\text{CH}_3$  and  $\text{CHCl}_3$  respectively, a collision yield of  $10^{-7}$  is obtained for reaction (12) at  $30^\circ\text{C}$ . The upper limit for  $E_{12}$  is then found to be  $9.6 \text{ kcal. per mole}$  corresponding to a steric factor of unity, or  $E_{12} = 6.8 \text{ kcal. per mole}$  if  $P_{12} = 10^{-2}$ . Since at  $150^\circ$   $k_{12}/k_2^{1/2} \ll \gamma_{\text{CHCl}_3}$ , a comparison of the total effect observed at  $150^\circ$  and the inhibited effect at  $30^\circ$  shows that  $E_{12}$  is smaller than about  $8 \text{ kcal. per mole}$ . It is thus evident that the previously reported (7) trend in the values of activation energies for hydrogen abstraction reactions for the series of chlorinated methanes is at least quali-

tatively correct. A hydrogen atom is more easily abstracted as the number of chlorine atoms substituted in the molecule of methane increases.

Further experiments are in progress with methyl chloride and other halogenated methanes.

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## THE PAPILIONACEOUS ALKALOIDS XVIII. LUPINUS SERICEUS PURSH.<sup>1</sup>

By LÉO MARION, NELSON J. LEONARD, AND BARRY P. MOORE<sup>2</sup>

### ABSTRACT

The plant investigated has been found to contain at least five alkaloids, three of which, *l*-sparteine, *d*-lupanine, and *d*- $\alpha$ -isolupanine, are of known structure. The remaining two are both isomeric with hydroxylupanine. One of these, lupilaxine, was identical with a base previously reported as occurring in *Lupinus laxus*. The other, lupanoline, appeared to be new. Both these alkaloids were shown by their infrared absorption spectra to contain, like hydroxylupanine, one hydroxyl group and one carbonyl.

It has been reported by Couch (3) that *Lupinus sericeus* Pursh. contains the alkaloids spathulatine and nonalupine. These, however, have since been shown to be identical and to consist of the monohydrate of pusilline monohydrochloride (4). In order to investigate the structure of pusilline a quantity of plant material tentatively identified as *L. sericeus* Pursh. was obtained through the courtesy of S. B. Penick and Company. Some doubt was raised concerning the identity of this plant when it was found to contain no pusilline. Couch had also investigated a closely related plant, *L. sericeus* var. *flexuosus* C. P. Smith, in which he found octalupine (2) later shown to be identical with hydroxylupanine (5). This base did not occur in the plant under investigation in which five different alkaloids have now been isolated. Owing to the impossibility so far of procuring more of this plant, and to the importance of the alkaloids found in it, the work is reported without further delay in spite of its uncertain identity.

Of the alkaloids isolated from this plant there are two (*l*-sparteine and *d*-lupanine) which are well known, one (*d*- $\alpha$ -isolupanine) which has been reported for the first time recently (7), and two which are isomeric with hydroxylupanine but not identical with it. It is noteworthy that *d*- $\alpha$ -isolupanine and matrine, which are isomeric, show a striking similarity in their properties and those of their salts, although the latter is now known to possess a quite different structure (8). One of the two isomers of hydroxylupanine has been shown by direct comparison (melting points and X-ray powder patterns) with an authentic sample to be identical with the "isomer of hydroxylupanine" previously isolated by Couch<sup>3</sup> from *Lupinus laxus* (1); it is therefore proposed to designate it as lupilaxine. Its infrared absorption spectrum showed absorption peaks at 3623 cm.<sup>-1</sup> and 1628 cm.<sup>-1</sup> indicative respectively of a hydroxyl group and a lactam carbonyl. Attempts to form an anhydro-base or a benzoyl derivative both failed.

The second isomer of hydroxylupanine found in this plant appears to be new and it is proposed to designate it as lupanoline. The bulk of it crystallized readily from an acetone solution of the crude mixture of alkaloids. It gave rise to a monohydrochloride and a methiodide. Although the melting points of the base

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<sup>2</sup> National Research Council of Canada Postdoctorate Fellow (1950-51).

<sup>3</sup> The alkaloid isolated from *L. laxus* was generously given to us by Dr. James F. Couch not long before his death, together with all his samples of lupine alkaloids.

and its monohydrochloride were practically the same as those of hydroxylupanine and its corresponding salt, admixture caused strong depressions. Furthermore, the infrared absorption spectra of the two bases were quite different although they offered some points of similarity. The spectrum of lupanoline showed absorption peaks at frequencies indicative of the presence of both a lactam carbonyl and a hydroxyl group. No functional derivative involving the hydroxyl group could be prepared.

Lupanoline is a derivative of  $\beta$ -isosparteine<sup>4</sup> and therefore the plant investigated affords the first example of one containing alkaloids derived from all three stereoisomers of the sparteine structure (6).

#### EXPERIMENTAL<sup>5</sup>

The plant was collected in the late blooming stage in the northern part of the State of Washington. Dried and ground it weighed 9308 gm. It was extracted with methanol and the extract concentrated on the steam bath. The concentrated extract was diluted with twice its volume of water, made acid to Congo red with hydrochloric acid, and heated on the steam bath for one hour. The mixture was cooled first to room temperature, then in the refrigerator, and filtered with suction. The insoluble cake was heated with dilute hydrochloric acid for one hour, cooled as before, and filtered. Both filtrates were combined and diluted with water; this caused the precipitation of a gum which was removed by filtration through filter-cel. This last filtrate was extracted repeatedly with large volumes of ether, alkalized with ammonia, and thoroughly extracted with chloroform. To the aqueous liquor was added a concentrated potassium hydroxide solution and the strongly basic liquor was again extracted with chloroform. The chloroform extracts were combined and the solvent distilled off under reduced pressure. To remove the last of the chloroform, a little methanol was added to the residue which was heated on the steam bath under reduced pressure. There was left a thick basic syrup (wt. 92.3 gm.) consisting of the crude alkaloids.

#### *Lupanoline*

The crude alkaloid syrup was dissolved in acetone and the solution allowed to stand in the refrigerator for several days during which a crystalline deposit gradually formed. The crystalline substance was filtered and recrystallized repeatedly from acetone from which it separated as colorless stout prisms, m.p. 174–176°.  $[\alpha]_D^{25} + 31^\circ \pm 1$  (*c.* 0.726 in ethanol),  $+ 64.4^\circ \pm 0.8$  (*c.* 0.88 in water). Calcd. for  $C_{15}H_{24}O_2N_2$ : C, 68.14; H, 9.15; N, 10.60. Found: C, 69.06, 68.93; H, 8.94, 8.75; N, 10.86, 10.78% (dried *in vacuo* at 100°). Sublimation did not alter the analytical results. When exposed to a moist atmosphere it formed a hydrate. In admixture with hydroxylupanine the melting point was markedly decreased. Wt. 6 gm.

A small quantity of lupanoline was dissolved in methanol and the solution made just acid to Congo red with methanolic hydrochloric acid. The solution was evaporated to dryness and the residue dissolved in boiling acetone from which the hydrochloride separated in beautiful colorless prisms. After one recrystallization

<sup>4</sup> Moore, B. P. and Marion, L. *Can. J. Chem.* 31: 187-92. 1953.

<sup>5</sup> All melting points are corrected.

from acetone-methanol it melted at 275–276°. Calcd. for  $C_{16}H_{24}O_2N_2 \cdot HCl$ : C, 59.90; H, 8.38; N, 9.32. Found: C, 60.49, 60.64; H, 8.27, 8.31; N, 9.42, 9.39%.

The methiodide, prepared by boiling the base with acetone and methyl iodide, was recrystallized from methanol. It consisted of long colorless needles, m.p. 230–231°. Calcd. for  $C_{16}H_{24}O_2N_2 \cdot CH_3I$ : C, 47.30; H, 6.70; N, 6.90. Found: C, 47.37, 47.21; H, 6.31, 6.43; N, 7.78, 7.74%.

The acetone mother liquor from which lupanoline had crystallized was evaporated to dryness and the residue dissolved in dilute hydrochloric acid. The acid solution was thoroughly extracted with ether, alkalized with strong aqueous potassium hydroxide, and extracted with chloroform, first in a separatory funnel and then in a continuous liquid-liquid extractor. The extracts were combined except for a quantity of the first separatory funnel extract which was evaporated to dryness and used for chromatographic separation. The bulk of the extract was distilled on the steam bath to remove most of the solvent while the last traces were removed by azeotropic distillation with methanol.

A quantity (3 gm.) of the crude base reserved for chromatography was dissolved in benzene (200 ml.) and the solution passed through a column of 60 gm. of alumina. The elution was made with (a) 200 ml. of benzene (10 fractions), (b) 300 ml. of 50–50 benzene-ether (7 fractions), (c) 200 ml. of ether (3 fractions), (d) 300 ml. of 95–5 ether-methanol (3 fractions), and (e) 200 ml. of 90–10 ether-methanol (3 fractions). Twenty-six fractions were thus collected. The first four fractions contained negligible quantities of base and were discarded. Fractions 5, 6, and 7 were combined (582 mgm.) and fractionated by distillation *in vacuo* into: fraction (a), b.p. 117–126° at 0.1 mm., faintly yellow oil; fraction (b), b.p. 125–145° at 0.1 mm., faintly yellow oil, and a residual reddish resin.

#### *l*-Sparteine

Fraction (a) was divided into two portions. One of these was added to methanolic picric acid and the crystalline picrate that separated was recrystallized from methanol. It consisted of fine yellow needles, m.p. 207–208° either alone or in admixture with *l*-sparteine dipicrate.

The second portion of fraction (a) was dissolved in methanol and perchloric acid was added dropwise to a phenolphthalein end point. A crystalline perchlorate separated which after two recrystallizations from methanol-ether consisted of colorless needles melting at 173° either alone or in admixture with *l*-sparteine perchlorate. In admixture with *d*-sparteine perchlorate it began to soften at 133° and melted over a wide range. *l*-Sparteine constitutes about 15% of the alkaloid mixture after removal of lupanoline.

#### *d*- $\alpha$ -Isolupanine

Fraction (b) from the distillation proved to consist of the same alkaloid, although less pure, as that obtained from fraction 8 of the chromatogram. The latter yielded a crystalline base which after two recrystallizations from petroleum ether consisted of colorless needles, m.p. 70–72°, unchanged when mixed with *d*- $\alpha$ -isolupanine (7). After purification through the perchlorate the base melted at 75–76°.  $[\alpha]_D^{26} + 39^\circ \pm 1$  (c, 0.774 in water). The infrared absorption spectrum of the purified base was exactly superposable on that of *d*- $\alpha$ -isolupanine (7).



The perchlorate of the base was prepared in methanol by the dropwise addition of 72% perchloric acid to a Congo red end point. Ether was then added to incipient cloudiness and the solution cooled in the refrigerator. The perchlorate after two recrystallizations from methanol-ether consisted of colorless elongated prisms, m.p. 239° (uncorr.) alone or in admixture with *d*- $\alpha$ -isolupanine perchlorate (6, 7).

A small quantity of the base was dissolved in ethanol-ether and the solution neutralized by the dropwise addition of concentrated hydrochloric acid. The solution was evaporated to dryness and the residue dissolved in ethanol-ether from which the hydrochloride separated as very small colorless prisms which softened at 148° and melted over a range.

The picrate of the base was prepared as previously described (7). It melted at 196–198° either alone or in admixture with *d*- $\alpha$ -isolupanine picrate.

To a solution of the base in absolute ethanol was added a solution of auric chloride in absolute ethanol containing a few drops of concentrated hydrochloric acid. On standing overnight the solution deposited the chloroaurate in clusters of elongated prisms which after two recrystallizations from absolute ethanol containing a few drops of hydrochloric acid melted at 195.5–196.5° when immersed at 175°.

The chloroplatinate was prepared from a solution of the base in absolute ethanol containing a few drops of hydrochloric acid by the addition of chloroplatinic acid. It consisted of short orange prisms, m.p. 241–243°.

Fractions 9 to 15 of the chromatogram also contained *d*- $\alpha$ -isolupanine. It was found that the alkaloid could be obtained pure when the base recovered from the chromatogram fractions was first washed with ether which dissolved the  $\alpha$ -isolupanine while a gummy impurity remained undissolved. The base recovered from the ether solution, after recrystallization from petroleum ether, consisted of pure *d*- $\alpha$ -isolupanine.

Fraction 22, eluted with ether containing 5% methanol, consisted after evaporation of the solvent of sticky crystals which crystallized from acetone as colorless stout prisms, m.p. 169–170°. In admixture with lupanoline (m.p. 174–176°) the melting point was unchanged. This fraction (500 mgm.) consisted mostly of lupanoline.

#### *d*-Lupanine

A quantity of the crude base was warmed with shaking with petroleum ether and the mixture allowed to settle. The clear solution was poured off and the residue warmed again repeatedly with fresh portions of petroleum ether until the residue left by evaporation of the solvent was negligible. The base recovered by evaporation of the combined petroleum ether extracts will be designated crude base *A* and the residue insoluble in petroleum ether, crude base *B*.

Crude base *A* was fractionated *in vacuo* into fraction I, b.p. 90°–100° at 0.15 mm., fraction II, b.p. 110°–140° at 0.15 mm., and a small undistilled residue. Fraction I consisted of *l*-sparteine. It was converted to the dipicrate, m.p. 208°, and to the perchlorate, m.p. 173°.

Fraction II was dissolved in methanol and the solution neutralized to Congo red by the dropwise addition of 72% perchloric acid. The perchlorate which



crystallized was filtered and recrystallized twice from methanol-ether, m.p. 197–198°. It was dissolved in water, the solution alkalinized with ammonia and exhaustively extracted with chloroform. The oily base recovered from the extract by evaporation of the solvent was redistilled; it boiled at 125–138° at 0.2 mm. Part of the distillate was dissolved in methanol and converted to the perchlorate as before. The crystalline perchlorate was filtered and recrystallized from methanol-ether from which it separated as colorless feathery aggregates, m.p. 211° either alone or in admixture with *d*-lupanine perchlorate.

The remainder of the distilled base was dissolved in water and a stoichiometrical proportion of ammonium thiocyanate in water was added. The resulting solution was evaporated to dryness and the residue crystallized from boiling acetone. When immersed in a bath at 155° the base thiocyanate liquefied but immediately resolidified and melted at 183°. When heated slowly from about 100°, it melted at 180°. Admixture with *d*-lupanine thiocyanate did not alter the melting point behavior.

The original mother liquor from the crystallization of *d*-lupanine perchlorate when allowed to evaporate slowly at room temperature deposited crystals which after two recrystallizations from methanol consisted of colorless prisms, m.p. 231–233°, unaltered by admixture with *d*- $\alpha$ -isolupanine perchlorate.

#### *Lupilaxine*

Crude alkaloid *B* which was insoluble in petroleum ether was distilled *in vacuo*. About two-thirds of the material distilled at 140–180° at 0.3 mm., as a pale yellow oil while the residue consisted of a dark resin. Since the distillate failed to crystallize from acetone it was dissolved in benzene and chromatographed on alumina. It was eluted with benzene, benzene-ether, ether, and finally ether containing 5% of methanol. The base obtained by evaporation of the ether-methanol eluate crystallized; that obtained from the ether fraction partly crystallized on seeding with the first crystals but consisted in part of an oily product. The oily base when distilled *in vacuo* afforded a fraction, b.p. 170–175° at 0.4 mm., which soon crystallized. The benzene eluate also yielded a quantity of this crystalline base. The various portions of crystalline base were combined and recrystallized from acetone from which it separated as colorless prisms, m.p. 175° (wt. 0.85 gm.). This base gave large depressions in melting point when mixed with lupanoline or with hydroxylupanine. However, the melting point was unaltered when the base was mixed with a sample of the isomer of hydroxylupanine isolated by Couch (1) from *Lupinus latus* Rydb. The infrared absorption spectrum of the base showed the presence of a hydroxyl group and a carbonyl group (absorption bands at 3623 cm.<sup>-1</sup> and 1628 cm.<sup>-1</sup>). The X-ray powder patterns of lupilaxine and of the "isomer of hydroxylupanine" isolated by Couch were exactly superposable.

#### ACKNOWLEDGMENT

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Division of these laboratories for the X-ray powder patterns.

One of us (N. J. L.) wishes to thank the Rockefeller Foundation for a fellowship and the National Research Council of Canada for the generous hospitality afforded during the summer of 1950.

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# THE PAPILIONACEOUS ALKALOIDS

## XIX. THE STRUCTURE OF LUPANOLINE<sup>1</sup>

BY BARRY P. MOORE<sup>2</sup> AND LÉO MARION

### ABSTRACT

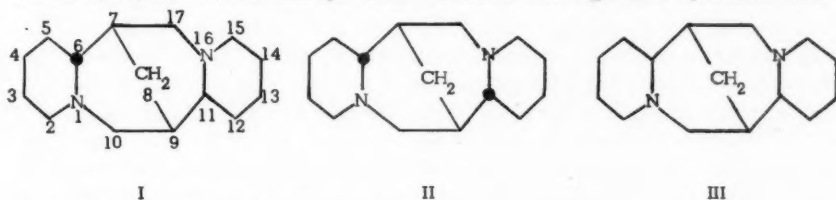
Lupanoline which contains both a carbonyl and a hydroxyl group was reduced by lithium aluminum hydride to  $\beta$ -isosparteine. The identity of  $\beta$ -isosparteine was established by its conversion into sparteine by dehydrogenation and subsequent rehydrogenation. Attempts to acylate lupanoline resulted in the formation of anhydrolupanoline which was hydrogenated to dihydroanhydrolupanoline, a compound also obtained by the direct oxidation of  $\beta$ -isosparteine. It is concluded that lupanoline is 2-hydroxy-17-oxo- $\beta$ -isosparteine.

The alkaloid lupanoline, the occurrence of which has been reported recently (4), is an isomer of hydroxylupanine,  $C_{16}H_{24}O_2N_2$ . It behaves as a monoacidic base, giving rise to a monohydrochloride and a monomethiodide so that one of its two nitrogens is neutral. The infrared absorption spectrum of lupanoline shows absorption peaks at  $3591\text{ cm}^{-1}$  and  $1624\text{ cm}^{-1}$  corresponding to a hydroxyl group and a carbonyl respectively, and thus accounting for both oxygen atoms.

The carbonyl group of lupanoline is inert towards the usual ketone reagents and is apparently lactamic in character. The presence of a nonbasic nitrogen in the molecule and the location of the carbonyl absorption band in the infrared spectrum support this conclusion. The hydroxyl group could not be acylated. Under the wide variety of conditions investigated, the group was invariably eliminated with the formation of anhydrolupanoline,  $C_{15}H_{22}ON_2$ , a substance which regenerated lupanoline on treatment with mineral acids. This characteristic behavior of the hydroxyl group of lupanoline is noteworthy.

Reduction of lupanoline with lithium aluminum hydride in dioxane proceeded smoothly, leading to the complete removal of oxygen from the molecule with the production of an oily base,  $C_{15}H_{26}N_2$ . Hence the reaction involved not only the conversion of the carbonyl to a methylene group thus confirming its lactamic nature, but also involved the reductive elimination of the hydroxyl. The reduction product was identical neither with sparteine nor with  $\alpha$ -isosparteine although isomeric with them, and it appeared possible that it might be the so far unknown  $\beta$ -isosparteine.

It has already been shown (2, 3) that of the three possible stereoisomeric forms I, II, and III of the sparteine structure two represent sparteine and



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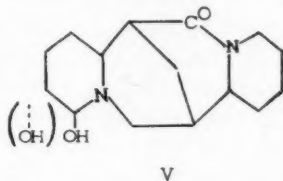
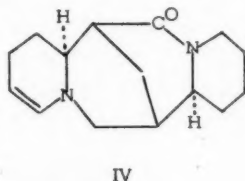
<sup>2</sup> National Research Council of Canada Postdoctoral Fellow, 1950-1951.

$\alpha$ -isosparteine. In formula I which represents sparteine the C<sub>6</sub>-hydrogen is *cis* to the 7,9-methylene bridge whereas the C<sub>11</sub>-hydrogen is *trans*. In  $\alpha$ -isosparteine (II) both the C<sub>6</sub>-hydrogen and the C<sub>11</sub>-hydrogen are *cis* to the 7,9-methylene bridge (3). This structure which was assigned to  $\alpha$ -isosparteine on the basis of hydrogenation results (3) has since been confirmed by a detailed X-ray crystallographic examination (5).  $\beta$ -Isosparteine should then be represented by III in which both the C<sub>6</sub>-hydrogen and the C<sub>11</sub>-hydrogen are *trans* to the 7,9-methylene bridge. Winterfeld and Rauch (6) converted sparteine into  $\alpha$ -isosparteine by dehydrogenation with mercuric acetate, which removed two moles of hydrogen, and subsequent rehydrogenation. This conversion must therefore have resulted from the elimination of the *cis* C<sub>6</sub>-hydrogen and the *trans* C<sub>11</sub>-hydrogen, and their replacement both in the *cis* position. The same reaction should convert  $\beta$ -isosparteine to  $\alpha$ -isosparteine if it removed two moles of hydrogen, or to sparteine if it removed only one mole. The reduction product of lupanoline was therefore oxidized with mercuric acetate and immediately rehydrogenated catalytically. The quantity of precipitated mercurous acetate corresponded to one mole of hydrogen, and the base obtained after rehydrogenation was identical with *l*-sparteine. Hence, the reduction product is  $\beta$ -isosparteine and lupanoline is an hydroxy-oxo- $\beta$ -isosparteine.

Information as to the positions of the substituents in lupanoline has been derived from a study of anhydrolupanoline and its derivatives. Anhydrolupanoline was readily hydrogenated over Adams' catalyst to yield the saturated dihydroanhydrolupanoline, C<sub>15</sub>H<sub>24</sub>ON<sub>2</sub>, which had the composition of an oxosparteine, and could be prepared by the direct oxidation of  $\beta$ -isosparteine with alkaline potassium ferricyanide. By analogy with the similar oxidation of sparteine to oxosparteine, it is reasonable to assume oxidation to have occurred at the 17-position (or equivalent 10-position) in  $\beta$ -isosparteine (III). Dihydroanhydrolupanoline could also be obtained directly from lupanoline by catalytic hydrogenation in acid solution. The failure of the carbonyl to reduce under these conditions also militates in favor of its occupying the 17-position.

On reduction with lithium aluminum hydride, anhydrolupanoline afforded an unstable oily dehydro-base which was readily converted to  $\beta$ -isosparteine on catalytic hydrogenation. No stereochemical inversion, therefore, is involved in the series: lupanoline  $\rightarrow$  anhydrolupanoline  $\rightarrow$  dehydro- $\beta$ -isosparteine  $\rightarrow$   $\beta$ -isosparteine. Thus it is clear that, if the possibility of migration be discounted, the double bond of anhydrolupanoline and the hydroxyl group of lupanoline itself cannot involve an angular position. The characteristic properties of the hydroxyl group (ready elimination and reduction) suggest association with a basic center in the form of an aldehyde-ammonia. The reductive elimination of the hydroxyl by lithium aluminum hydride particularly is indicative of such a grouping. The formulation of lupanoline as an aldehyde-ammonia is supported by the facile rehydration of anhydrolupanoline to the parent alkaloid under the influence of mineral acid. Thus anhydrolupanoline would appear to be best formulated as IV and lupanoline as 2-hydroxy-17-oxo- $\beta$ -

isosparteine (V). An alternative formulation with a 10-hydroxyl group is



considered unlikely as the preparation of an anhydro-base from such a system would require the formation of a double bond at a bridge head, in violation of Bredt's rule. Whether the hydroxyl group at the 2-position is *cis* or *trans* is not yet known.

Lupanoline is thus the first alkaloid known to be derived from  $\beta$ -isosparteine. The hitherto unknown  $\beta$ -isosparteine prepared from it is an oil which like sparteine discolors on standing in air. It has not yet been possible to induce its picrate to crystallize, but like its two other stereoisomers it forms a crystalline perchlorate. For the infrared absorption spectrum of  $\beta$ -isosparteine and the spectra of its two stereoisomers see Fig. 3, Ref. (1).

Anhydrolupanoline and its hydrogenation product are isomers of monspessulanine and dihydromonspessulanine, and their melting points are about the same. However, admixture with authentic samples of these two bases caused depressions in the melting points, and the infrared absorption spectra were quite distinct from those of the two derivatives of lupanoline. The sample of monspessulanine was kindly given to us by Dr. E. P. White.

#### EXPERIMENTAL<sup>3</sup>

Lupanoline has been described previously (4). It melts at 174–176° and has  $[\alpha]_D^{26} + 64.4^\circ \pm 0.8$  (c, 0.88 in water). It forms a monohydrochloride, m.p. 275–276°, and a methiodide,  $C_{15}H_{24}O_2N_2 \cdot CH_3I$ , m.p. 230–231°. Its infrared absorption spectrum showed absorption peaks at 1624  $cm^{-1}$  and 3593  $cm^{-1}$  indicating the presence of a lactam carbonyl and a hydroxyl group respectively.

#### *Reduction of Lupanoline with Lithium Aluminum Hydride*

A solution of lupanoline (250 mgm.) in pure, dry dioxane (5 ml.) was treated with an ethereal solution of lithium aluminum hydride (3 ml., 0.016 gm. per mole, 1.3 moles) and the mixture refluxed in an atmosphere of nitrogen for four hours (the ether being allowed to escape). The mixture was cooled, treated with a little ethanol to destroy excess hydride, and then acidified with dilute hydrochloric acid. The solution was freed from nonbasic impurities by extraction with ether and alkalized with aqueous potassium hydroxide. The liberated base was extracted with ether and after evaporation of the extract was distilled *in vacuo*. The main fraction afforded  $\beta$ -isosparteine as a colorless oil, b.p. 110–120° (air bath) at 1 mm., which gave a crystalline diperchlorate crystallizing from methanol as colorless prisms, m.p. 255° (dec.). Calcd. for

<sup>3</sup> All melting points are corrected.

$C_{15}H_{26}N_2 \cdot 2HClO_4$ : C, 41.38; H, 6.48; N, 6.43. Found: C, 41.36, 41.22; H, 6.28, 6.55; N, 5.95%.

#### *Conversion of $\beta$ -Isosparteine to Sparteine*

A mixture of  $\beta$ -isosparteine (234 mgm., 1 mM.), 5% aqueous acetic acid (35 ml.), and mercuric acetate (2.5 gm., 8 mM.) was stirred under an atmosphere of nitrogen and heated on a water bath for three hours. The mixture was cooled and the crystalline mercurous acetate collected by filtration (1 mM. obtained  $\equiv$  2H). The clear aqueous solution was saturated with hydrogen sulphide and the precipitated mercuric sulphide filtered off. The aqueous filtrate was evaporated *in vacuo* to a small bulk, diluted with water, and evaporated again in order to ensure complete removal of hydrogen sulphide. The concentrated liquor was alkalinized with aqueous sodium hydroxide and extracted with chloroform. The washed chloroform extract was dried rapidly over anhydrous potassium carbonate, filtered, added to glacial acetic acid (7 ml.), and the resulting solution heated on a steam bath until all the chloroform had evaporated. Since the dehydro-base thus obtained was not stable it was not isolated from the acetic acid concentrate, but was hydrogenated directly over fresh Adams' catalyst for 12 hr. The hydrogen uptake (ca. 40 ml.) corresponded approximately to 2H. After filtration the solution was alkalinized with aqueous sodium hydroxide and extracted with chloroform. Evaporation of the chloroform extract yielded a base which was distilled; it consisted of a colorless oil (80 mgm.), boiling at 0.4 mm. at an air bath temperature of 95–105°, which did not crystallize. The dipicrate of this base, which crystallized from a solution of the components in methanol, was recrystallized from methanol. It consisted of bright yellow needles, m.p. 209°, undepressed in admixture with authentic *l*-sparteine dipicrate. The infrared absorption spectrum taken as a Nujol mull was identical with that of *l*-sparteine dipicrate measured under the same conditions.

#### *Anhydrolupanoline*

A solution of lupanoline (500 mgm.) in purified acetic anhydride (3 ml.) was refluxed for one hour, cooled, and treated with cold water. It was extracted twice with ether and alkalinized with aqueous sodium hydroxide. The liberated base was extracted with ether, recovered from the extract by evaporation of the solvent, and distilled *in vacuo*. Anhydrolupanoline was thus obtained as a colorless oil, b.p. 145–150° at 0.1 mm., which crystallized readily from ligroin (60–80°) in colorless prisms, m.p. 94–95°, yield 315 mgm. Recrystallization did not alter the melting point.  $[\alpha]_D^{24} = 43.1^\circ \pm 1$  (*c*, 0.58 in water). Calcd. for  $C_{16}H_{22}ON_2$ : C, 73.13; H, 9.01. Found: C, 73.15, 73.30; H, 9.03, 8.85%. In admixture with monspessulanine (m.p. 100°) of which it is an isomer it melted at 65–70°. Admixture with apyllidine also caused a depression.

Anhydrolupanoline formed a perchlorate in the usual way which separated from methanol as colorless, elongated prisms, m.p. 252–253° (dec.).  $[\alpha]_D^{26} = 57^\circ \pm 1$  (*c*, 0.912 in water). Calcd. for  $C_{16}H_{22}ON_2 \cdot HClO_4$ : C, 51.94; H, 6.68; N, 8.08. Found: C, 51.77; H, 6.68; N, 7.71%.



*Dihydroanhydrolupanoline*

Anhydrolupanoline (100 mgm.) was hydrogenated in ethanol (10 ml.) at room temperature and atmospheric pressure in the presence of freshly prepared Adams' catalyst (50 mgm.). Hydrogenation proceeded rapidly and was essentially complete within 15 min. After one hour (hydrogen uptake ca. 15 ml.) the mixture was filtered and the filtrate evaporated on a steam bath. The residual dihydroanhydrolupanoline crystallized readily from ligroin (60–80°) in colorless sheaves, m.p. 103–104° (yield 70 mgm.). Calcd. for  $C_{15}H_{24}ON_2$ : C, 72.51; H, 9.74; N, 11.28. Found: C, 72.56, 72.78; H, 9.59, 9.58; N, 11.15%. The infrared absorption spectrum of the base was not identical with that of the isomeric dihydromonspessulanine.<sup>4</sup>

A small quantity of the base was dissolved in methanol and the solution made just acid to Congo red by the dropwise addition of 67% perchloric acid. On standing the solution deposited a crystalline perchlorate which after several recrystallizations from methanol consisted of colorless prisms, m.p. 224–225°. Calcd. for  $C_{15}H_{24}ON_2 \cdot HClO_4$ : C, 51.65; H, 7.17. Found: C, 51.61, 51.72; H, 6.84, 6.75%.

*Action of Mineral Acid on Anhydrolupanoline*

A solution of anhydrolupanoline (100 mgm.) in *N* hydrochloric acid was heated to 100° for two hours, then cooled and alkalinized with aqueous sodium hydroxide. The product, isolated by extraction with chloroform, crystallized from ligroin (60–80°) in colorless rhombs, m.p. 163–167° (65 mgm.). Two recrystallizations raised the melting point to 169–170° (with previous shrinking). The material gave no depression in melting point on admixture with pure lupanoline. Calcd. for  $C_{15}H_{24}O_2N_2$ : C, 68.14; H, 9.15; N, 10.60. Found: C, 67.53, 67.83; H, 9.27, 8.91; N, 10.50%. This base was converted by the action of lithium aluminum hydride into  $\beta$ -isosparteine which was identified as its perchlorate. It is thus identical with lupanoline but probably contains a trace of an isomer sufficient to cause the unsharp melting point behavior.

*Reduction of Anhydrolupanoline with Lithium Aluminum Hydride*

Anhydrolupanoline (100 mgm.) in dry ether (10 ml.) was treated with ethereal lithium aluminum hydride (1 ml., 0.015 gm. per ml.) and the mixture refluxed for four hours. Water was then added cautiously, followed by aqueous sodium hydroxide, and the product isolated with ether. A portion of the oily base thus obtained decomposed on attempted distillation *in vacuo* and formed an oily perchlorate. The remainder of the product was hydrogenated in acetic acid solution over fresh Adams' catalyst for 12 hr. The basic product, isolated in the usual way, distilled as an oil, b.p. 110–120° (air bath) at 0.2 mm. The diperchlorate, m.p. 255° (dec.), proved to be identical with that of  $\beta$ -isosparteine.

*Oxo- $\beta$ -isosparteine*

$\beta$ -Isosparteine (freshly distilled, 130 mgm.) was shaken vigorously with

<sup>4</sup> Dihydroanhydrolupanoline is also obtainable directly by catalytic hydrogenation of lupanoline. This experiment as well as the comparison with dihydromonspessulanine was carried out by Dr. Bryce Douglas.

cold water (25 ml.), the resulting suspension treated with a solution of potassium ferricyanide (660 mgm.) and potassium hydroxide (60 mgm.) in water (10 ml.) and shaken mechanically for one hour. The resulting straw-colored solution was allowed to stand overnight at room temperature, and then extracted with chloroform. The crude base obtained by evaporation of the chloroform extract was distilled *in vacuo* and afforded a pale yellow oil (35 mgm.), b.p. 135–140° (air bath) at 0.5 mm., together with a considerable nonvolatile residue. The distillate was dissolved in ligroin (60–80°), the solution filtered, evaporated to a small bulk, and allowed to stand. Oxo- $\beta$ -sparteine crystallized which after recrystallization from ligroin (60–80°) consisted of colorless sheaves, m.p. 103–104°, undepressed on admixture with a specimen of dihydroanhydrolupanoline.

#### ACKNOWLEDGMENT

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# THE ESSENTIAL OIL OF *ABIES BALSAMEA* (L.) MILL.<sup>1</sup>

By A. C. SHAW

## ABSTRACT

Foliage of *Abies balsamea* yielded 0.591% of an essential oil comprised of *l*- $\beta$ -pinene, *l*-bornyl acetate, *d*- $\Delta^3$ -carene, *l*-limonene, *l*- $\alpha$ -pinene, *l*-camphene, myrcene, santene, tricyclene, *l*-camphor, *l*-borneol, *d*-canadene, bisabolene, terpinolene, and a compound related to S-guiazulene.

## INTRODUCTION

*Abies balsamea* (L.) Mill., or balsam fir, is one of the most prolific species in Eastern and Central Canada. It ranges from the Atlantic coast of Canada westward and northward to northeastern Alberta, and is cut extensively for both pulp and lumber. The essential oil of *A. balsamea* has been reported (4) to contain 17.6% bornyl acetate, and probably *l*- $\alpha$ -pinene. More recently an essential oil prepared from Canada balsam (12) was reported to contain *l*- $\alpha$ -pinene, *l*- $\beta$ -pinene, and *l*- $\beta$ -phellandrene. However it is probable that this latter oil, since it had an ester value of only 2.24, was actually a turpentine oil of balsam fir, and that the needle oil of *A. balsamea* has not previously been examined in detail.

## EXPERIMENTAL

### *Preparation of the Essential Oil*

Steam at 90 p.s.i. was admitted at the rate of 10 lb. per min. for six hours, to a cylindrical wooden still at atmospheric pressure containing 2960 lb. of foliage and terminal branches of *A. balsamea* which were collected and distilled in April, 1948. Because of heat losses and the large heat capacity of the charge, effluent steam from the still was in general saturated, and heat-sensitive compounds were not exposed to temperatures much in excess of 100°. The oil, obtained in a yield of 0.591%, had the following properties:  $\eta_D^{20}$  1.4737,  $d_4^{20}$  0.8825,  $\alpha_D^{30}$  - 18.8°, acid value 0.23, ester value 52.4, ester value after acetylation 77.2, aldehyde and ketone value, nil.

### *Fractional Distillation of the Oil*

The oil was fractionated according to the scheme shown in Fig. 1. The preliminary distillation, yielding the hydrocarbon, oxygen compound, and sesquiterpene fractions, was carried out without reflux through an unpacked column. The sesquiterpenes and higher boiling compounds were fractionated through a 1-ft., 25 mm. I.D. Podbielniak column at 10 mm. pressure, 3 mm. back pressure, and reflux ratio 15/1. For all other distillations a 6-ft., 25 mm. I.D. Podbielniak column was employed at a reflux ratio of 20/1, and 20 mm. back pressure. The properties of the fractions obtained are shown in Figs. 2 and 3. In Fig. 2 the fractions judged to be most nearly homogeneous were assigned letters *a* to *m*.

### *Identification of Constituents<sup>2</sup>*

The oil was found to be qualitatively very similar in composition to oil of *Picea*

<sup>1</sup> Manuscript received in original form December 21, 1951 and, as revised, November 17, 1952. Contribution from Ontario Research Foundation, 43 Queen's Park, Toronto 5, Ontario.

<sup>2</sup> Except where otherwise noted, melting points were determined with a Kofler micro hot stage. The identification of all solid derivatives was confirmed by determination of their melting points in admixture with authentic samples.

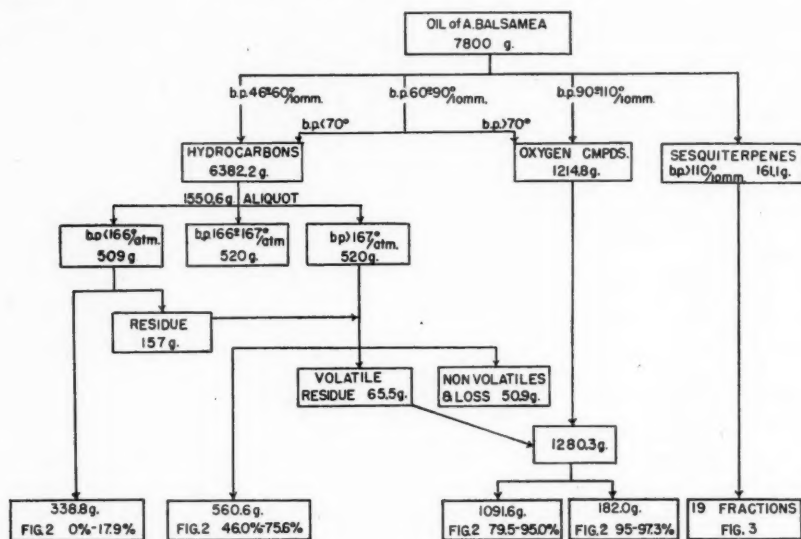


FIG. 1. Fractional distillation of the oil.

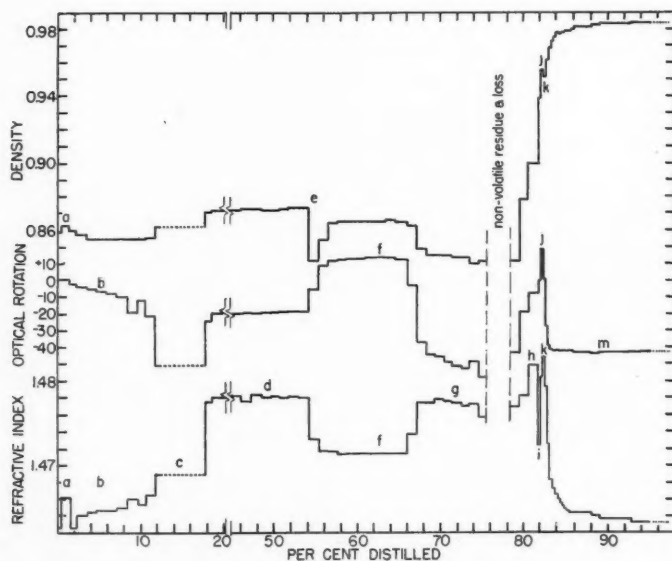


FIG. 2. Properties of fractions boiling below 110°C. at 10 mm.

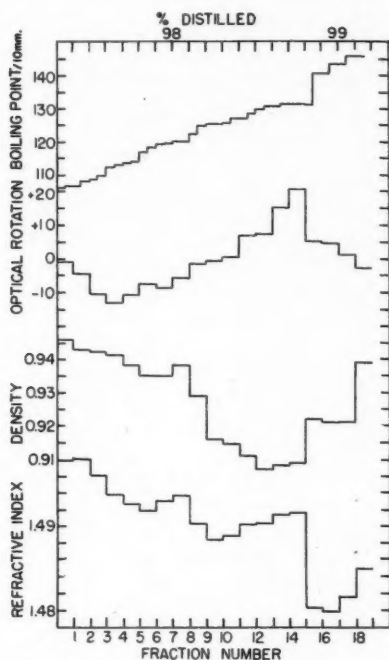


FIG. 3. Properties of fractions boiling above 110°C. at 10 mm.

*mariana* (Mill.) B.S.P. With the exception of the sesquiterpene compounds all constituents were identified by methods described in a previous communication (9) dealing with oil of *P. mariana*.

#### *Santene and Tricyclene*

Fraction *a* was redistilled at atmospheric pressure, 0.5 mm. back pressure, and reflux ratio 40/1, through the 1-ft. Podbielniak column, to separate two optically inactive compounds, b.p. 139.5°C.,  $\eta_D^{20}$  1.4664,  $d_4^{20}$  0.862, and b.p. 151.5–153.0°,  $\eta_D^{20}$  1.4610,  $d_4^{20}$  0.866. The former, when treated with nitrous acid according to Müller (7), gave santene nitrosite m.p. 123–125°C. in 30.9% of theoretical yield. The latter compound was treated for one hour with 10 volumes of 10% aqueous permanganate at 100°C. to remove unsaturated impurities. Unchanged tricyclene, recovered in a current of steam, melted at 65°C. after crystallization from methanol and sublimation from sodium.

#### *dl- $\alpha$ -Pinene*

By permanganate oxidation (2) of 39.5 gm. of fraction *b* ( $\eta_D^{20}$  1.4648,  $d_4^{20}$  0.855,  $\alpha_D - 6.03^\circ$ ) 15.02 gm. of crude acids were obtained from which, by repeated crystallization from benzene–hexane mixtures, *dl*-pinonic acid m.p. 104–106°C.,  $(\alpha)_D^{25} - 0.18^\circ$  ( $c = 10.88$ , chloroform) was separated.

*l*-Camphene

Fraction *c* was a solid, m.p. 43°C., b.p. 160.5°C., ( $\alpha$ )<sub>D</sub> - 91.3° (*c* = 10.33, benzene). Hydration of 5.0 gm. of this material with Bertram-Walbaum reagent (1) yielded 5.56 gm. of isoborneol, which after crystallization from hexane, melted at 210–213°C. in a sealed capillary.

*l*- $\beta$ -Pinene

Twenty grams of fraction *d* (b.p. 166.9°C. (758 mm.),  $\eta_D^{20}$  1.4783,  $d_4^{20}$  0.870,  $\alpha_D$  - 19.54°) in 200 ml. of acetone was cooled in a freezing mixture, and 48 gm. of powdered potassium permanganate added with mechanical stirring over 16 hr. The manganese dioxide was removed, washed with acetone, and finally extracted six times with 100 ml. volumes of boiling water. When concentrated under reduced pressure to a volume of 75 ml., the aqueous extracts deposited 2.60 gm. of a sparingly soluble potassium salt. The salt was recrystallized from water, and the free acid extracted from an acidified aqueous solution with ether. By crystallization from benzene-hexane mixtures, small needles of *l*-nopinic acid m.p. 126–127°C., ( $\alpha$ )<sub>D</sub><sup>25</sup> - 15.2° (*c* = 6.12, ether) were obtained.

*Myrcene*

Refractionation of fraction *e* gave a liquid b.p. 167.5–168°C. (751 mm.),  $\eta_D^{20}$  1.4720,  $d_4^{20}$  0.820,  $\alpha_D$  - 4.60° which reacted with maleic anhydride to give in 39% yield an adduct, m.p. 34–35°C., b.p. 145–155°C. (0.4 mm.). *cis*-4-Isohexenyl- $\Delta^4$ -tetrahydrophthalic acid m.p. 123–125°C. was obtained by saponification of the adduct.

*d*- $\Delta^3$ -Carene

Five grams of fraction *f* (b.p. 172.0°,  $\eta_D^{20}$  1.4715,  $d_4^{20}$  0.865,  $\alpha_D$  + 14.00) was mixed with amyl nitrite (4 gm.) and glacial acetic acid (2 ml.), and to this mixture 3.5 ml. of concentrated nitric acid was added dropwise with ice cooling. After 15 min. the product was precipitated with 10 ml. of methanol. After several precipitations from warm chloroform with methanol, *d*- $\Delta^3$ -carene nitrosate (0.45 gm.), m.p. 146°C. with decomposition, was obtained.

*l*-Limonene

Bromination of 10 gm. of fraction *g* (b.p. 177.9°C.,  $\eta_D^{20}$  1.4775,  $d_4^{20}$  0.844,  $\alpha_D$  - 51.33) in isoamyl alcohol-ether solution yielded 7.2 gm. of solid bromides, which were crystallized from ethanol to give 1.6 gm. of *dl*-limonene tetrabromide. The mother liquors, after concentration and removal of a further quantity of racemate, deposited at - 70°C. 150 mgm. of *l*-limonene tetrabromide m.p. 102–104°C.

*Examination of Fractions h and i*

Fraction *h* (10 gm.) was allowed to stand for one week in an ethanol solution containing excess semicarbazide hydrochloride and sodium acetate. Nonketonic compounds (4.6 gm.) were separated from the crude semicarbazones (2.9 gm.) by steam distillation. The crude semicarbazones deposited 0.69 gm. of crystalline material m.p. 231–233°C. from ethanol, which when mixed with an equal weight of oxalic acid and steam distilled was decomposed to yield 0.02 gm. of *dl*-camphor



m.p. 178–179°C.; 2,4-dinitrophenyl hydrazone m.p. 167–169°C.

The nonketonic portion was treated with *n*-butyl borate as previously described (9), and liberated *n*-butyl alcohol and nonalcoholic compounds separated from the borate esters (0.65 mgm.) by distillation. By bromination of this distillate 0.11 gm. of terpinolene tetrabromide m.p. 115–116°C. was obtained.

Fraction *i* (15.5 gm.) treated in an analogous manner gave 6.45 gm. of crude semicarbazones yielding camphor with  $(\alpha)_D - 24.0^\circ$  ( $c = 4.99$ , ethanol) on hydrolysis, and 10.2 gm. of nonketonic compounds from which 4.5 gm. of borate esters were prepared. The alcohol, regenerated by saponification of the combined borate esters of fractions *h* and *i*, boiled at 82–83°C. (10 mm.),  $\eta_D^{20}$  1.4743, and formed a *p*-nitrobenzoate (0.05 gm. from 0.6 gm.) m.p. 105–107°C.,  $(\alpha)_D + 21.02^\circ$  ( $c = 0.76$ , ethanol).

#### Examination of Fractions *j* and *k*

Fraction *j* (10.4 gm.) yielded 1.9 gm. of semicarbazone and 8.7 gm. of nonketonic oil. From fraction *k* (13.7 gm.), 4.10 gm. of semicarbazone and 9.6 gm. of nonketonic compounds were obtained. The semicarbazones did not crystallize well from common solvents. Fraction *k* semicarbazones were crystallized from acetonitrile to give 0.43 gm. of a crystalline compound m.p. 205–212°C. The acetonitrile insoluble semicarbazones were crystallized from nitromethane, and 0.36 gm. of a compound m.p. 214–219°C. obtained. Too little material was available to allow crystallization to constant melting point. As the semicarbazones were not hydrolyzed by the action of 1*N* sulphuric acid or oxalic acid at 100°C., the ketone was not isolated or identified.

The combined alcohols of fractions *j* and *k* (9.66 gm.), isolated by means of their borate esters, deposited 0.86 gm. of solid borneol when cooled. The liquid portion, b.p. 91.5–96°C. (12 mm.),  $\eta_D^{20}$  1.4848,  $d_4^{20}$  0.952,  $\alpha_D - 12.34^\circ$ , yielded only derivatives of borneol when treated with *p*-nitrobenzoyl chloride,  $\alpha$ -naphthyl isocyanate, or phenylisocyanate, and failed to form a solid hydrochloride. This fraction then consists of a mixture of borneol, and an unidentified tertiary alcohol.

#### Bornyl Acetate

Forty grams of fraction *m* ( $\eta_D^{20}$  1.4649,  $d_4^{20}$  0.980,  $\alpha_D - 43.98^\circ$ ) were saponified as previously described (10); and 33.5 gm. of borneol, m.p. 206–207°C.,  $(\alpha)_D - 39.02^\circ$  ( $c = 10.89$ , toluene), and 15 gm. of crude potassium salts obtained. A 20% aqueous solution of 5 gm. of the salts was allowed to percolate through 50 gm. of Amberlite resin IR-120, (hydrogen cycle) and the eluate refluxed briefly to remove carbon dioxide. The Duclaux constants of this solution were 7.0, 7.3, and 7.7, indicating the absence of significant amounts of volatile acids other than acetic. From 4.0 gm. of the salt, 0.5 gm. of acetanilide m.p. 114–115°C. was prepared.

#### Dehydrogenation of the Sesquiterpene Fractions

Only those fractions which appeared to contain a maximum concentration of one constituent were examined. Fractions 4, 6, 9, 10, 15, 17, and 18 were dehydrogenated as follows: A mixture of 1.0 gm. of the oil and 0.10 gm. of palladium-charcoal catalyst (6) was heated under nitrogen, at 200°C. for two hours, then at 245°C. for six hours. The reaction mixture was diluted with

hexane, filtered, and the filtrate extracted with 5% aqueous sodium hydroxide. Hexane was removed from the extracted filtrate and the residue in 10 ml. of ethanol was refluxed for 15 min. with 1.0 gm. of picric acid. Cadalene-picric acid addition compound m.p. 116–117°C. was obtained in a yield of 700 mgm. from fraction 15, 296 mgm. from fraction 10, and a few crystals from fraction 9. Fraction 17 gave a black product which apparently decomposed during recrystallization as only picric acid m.p. 121°C. was recovered. No picrate forming substances were obtained from fractions 4, 6, or 18.

A further quantity of fraction 17 dehydrogenation product in six volumes of hexane was extracted with two 1-ml. volumes of 85% phosphoric acid at 0°C. as described by Sherndal (11). The phosphoric acid was exhaustively extracted with hexane, diluted with ice water, and extracted with ether. Steam distillation of the ether extract gave 23 mgm. of an intensely blue oil, which when refluxed with 0.5 ml. of ethanol and the calculated weight of *sym*-trinitrobenzene formed black silky needles of *S*-guiaiazulene-trinitrobenzene addition compound m.p. 145–147°C. The authentic sample of *S*-guiaiazulene was prepared by dehydrogenation of the fraction b.p. 120°–130°C. (10 mm.) of gurjun balsam oil.

#### *Sesquiterpene Hydrochlorides*

A solution of 2.4 gm. of fraction 14 in 2.5 ml. of ethyl ether was saturated with dry hydrogen chloride at 0°C., and refrigerated overnight. After evaporation of the ether and removal of oily products on a cold porous tile, 1.1 gm. of hydrochlorides remained. The hydrochloride mixture was separated by crystallization from ethanol, and by mechanical means, into long needles of *l*-cadinene hydrochloride m.p. 138–139°C., and small platelets of bisabolene hydrochloride m.p. 80–81°C. We are indebted to Dr. E. Guenther for the gift of the distilled lime oil residues from which an authentic sample of bisabolene was isolated.

#### RESULTS AND DISCUSSION

The approximate composition of oil of *A. balsamea*, as estimated from the distillation data, is as follows:

TERPENES		OXYGEN COMPOUNDS	
<i>l</i> - $\beta$ -Pinene	36.1%	<i>l</i> -Bornyl acetate	14.6%
<i>d</i> - $\Delta^3$ -Carene	11.1%	<i>l</i> -Camphor	0.2%
<i>l</i> -Limonene	11.1%	<i>l</i> -Borneol	0.1%
<i>l</i> - $\alpha$ -Pinene	8.4%	Alcohol, b.p. 91.5–96°C. (12 mm.)	0.3%
<i>l</i> -Camphene	6.8%	Alcohol, b.p. 82–83°C. (10 mm.)	0.1%
Myrcene	2.3%	Unidentified ketones	0.2%
Santene	1.3%		
Tricyclene	1.3%	SESQUITERPENES	
Terpinolene	0.05%	<i>d</i> -Canadene	0.1%
Residue and loss	3.4%	Bisabolene	0.1%
Unaccounted for	2.3%	Compound related to <i>S</i> -guiaiazulene	Trace

The  $\beta$ -pinene content of 36.1% is among the highest recorded for any oil.

Tricyclene has now been identified in the needle oils of *P. mariana* (9), *Tsuga canadensis* (10), and *A. balsamea*. Its place as a normal constituent of coniferous oils appears well established.

If  $\beta$ -phellandrene were present as might be expected from the work of Smith and West (12), it would be found in fraction *f*. This fraction, however, failed to give a solid nitrosite with cold nitrous acid, indicating the absence of significant amounts of  $\alpha$ - or  $\beta$ -phellandrene.

In the absence of a reliable estimate of homogeneity it might be concluded that fraction 15 contains bisabolene, *l*-cadinene, and a third compound responsible for the dextrorotation of the fraction. However, cadalene was obtained in 32.5% yield from fraction 15. Linstead and co-workers (5) obtained cadalene in 50% yield by dehydrogenation of pure *l*-cadinene under comparable conditions. *l*-Cadinene hydrochloride, and a small amount of bisabolene hydrochloride, was obtained in 33.7% of the theoretical yield from fraction 15. Under the same conditions a 64.7% yield of hydrochloride was obtained from a pure *l*-cadinene regenerated from the hydrochloride. Thus if *l*-cadinene were present it would constitute, at a minimum, 60% of the fraction. Since bisabolene, which is optically inactive, is present in detectable quantities, the third constituent responsible for the dextrorotation of the fraction could not amount to more than about 30% of the fraction. Accepting the value of  $(\alpha)_D - 113.0^\circ$  for *l*-cadinene, the specific rotation of this third constituent would have to be about  $+300^\circ$  to account for the observed rotation,  $(\alpha)_D + 22.8^\circ$ , of fraction 15. Although a compound with this high rotation should be easily recognized, no sesquiterpene with a rotation approaching  $+300^\circ$  is known. More probably the dextrorotation is associated with the precursor of the cadalene and *l*-cadinene hydrochloride. Deussen (3) was first to isolate a dextrorotatory sesquiterpene which gave the hydrochloride of *l*-cadinene. He assigned the misleading name "*d*-cadinene" to this compound. Other workers who have isolated similar fractions have generally referred to the constituent as simply "cadinene" (8). Since the compound is actually a structural isomer of *l*-cadinene (the product of dehydrohalogenation of *l*-cadinene hydrochloride), this nomenclature is not satisfactory. The name "*d*-canadene" was suggested for the dextro sesquiterpene of oil of *Tsuga canadensis* and *P. mariana* (10). The sesquiterpene of *A. balsamea* is probably identical with that of these two latter species.

#### ACKNOWLEDGMENT

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